



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

= DEC 22 2003

Date: _____
From: Interdisciplinary Scientist/Pharmacist, Division of Dietary Supplement Programs
, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810
Subject: 75-Day Premarket Notification of New Dietary Ingredients
To: Dockets Management Branch, HFA-305

Subject of the Notification: ***Ganoderma lucidum* capsules**

Firm: Atlantic Medical Health Care, Inc. (Distributor)

Date Received by FDA: **March 26, 2003**

90-Day Date: **June 24, 2003**

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Marcia Chong for
Susan Aitken

95S-0316

RPT185

Atlantic Medical Health Care, Inc.

4115 Wilkens Ave. Suite 200 Baltimore, MD 21229

Tel.(410)737-9333 (301)816-9333 Fax: (410)737-2055

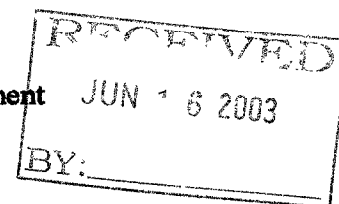
www.Atlantic-medical.com E-Mail: Atlantic-Medical1@comcast.net

June 12, 2003

TO: U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Nutritional Products, Labeling and Dietary Supplements

ATT: Ms. Victoria Lutwa
Fax.#301-436-2636

SUBJECT: Resubmission of documentation for Dietary Supplement
Ganoderma Lucidum capsule.



Dear Ms. Lutwak,

We are receipt of your fax-letter dated June 10, 2003. Ref. (Docket No. 95S-0316)

At this time, we are submitting additional documentation which we feel confident, will satisfy the FDA's requirements for Safety.

Until such time as we receive the proper clearance from the FDA, we will not offer this product for sale.

We are writing for you to keep our entire file confidential due to proprietary and trade secret material during the period this matter is under FDA consideration.

Dr. Xouling Xiong , is authorized by us to discuss and provide the FDA with material to satisfy any items that are in question.

I wish to thank you in advance for your attention to this request.

Very Sincerely Yours,


Ming LI V.P.

c/c

Dr. Y. Larry Xiong
Professor of Food Science and Nutrition
University of Kentucky
Lexington, KY 40546-0215
Phone: 859-257-3822
E-Mail: ylxiong@uky.edu



JUN 10 2003

Ms. Ming Li
Atlantic Medical Health Care, Inc.
4115 Wilkens Ave, Suite 200
Baltimore, Maryland 21229

Dear Ms. Li:

This is in response to your letter making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act). Your letter notifying the Food and Drug Administration (FDA) of your intent to market the substance *Ganoderma lucidum* capsule as a new dietary ingredient was filed March 26, 2003.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

The notification does not specifically identify the new dietary ingredient. The notification also does not contain a description of the dietary supplement that contains the new dietary ingredient including:

- (i) the level of the new dietary ingredient in the dietary supplement and
- (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or, if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

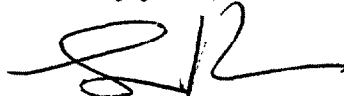
Your ingredient appears to be a preparation resulting from fermentation of mycelia of the fungus *Ganoderma lucidum*. The new dietary ingredient is not clearly identified by name or by composition. There appears to be no history of use of the ingredient. The information presented regarding safety does not clearly relate the materials tested to the material that is the subject of the notification.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that *Ganoderma lucidum*, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C.342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C.331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of March 26, 2003. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

Should you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-2375.

Sincerely yours,



Susan J. Walker, M.D.
Acting Division Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

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March 23, 2003

TO: U.S. Food and Drug Administration
Center for food Safety and Applied Nutrition

SUBJECT: Notification
Dietary Supplement - Ganoderma Lucidum capsule.

MAR 26 2003

Dear Sir / Madam,

We are submitting the documentation of the Ganoderma Lucidum capsule manufactured in China and imported by Atlantic Medical Health Care, Inc. accordance to the requirements specified in 21 CFR 190.6 for making the required notification.

The Ganoderma Lucidum (Chinese name: Linzhi, Japanese name: Reishi Mushroom) capsules are produced by submerged fermentation of Ganoderma Lucidum Mycelia. It contains no additives, such as preservatives, thickeners, or stabilizers. The product (Q/320217UAL 03-2002), has passed National Technical Appraisal in 1999 by the Ministry of Health P.R. of China, The dietary supplement sanitary license is WEISHIJIANZI (2001) NO. 0151 and the manufacture has been granted a patent in China.

The following 3 sets of documents are enclosed:

Documents Translated by:

Dr. Y. Larry Xiong

Professor of Food Science and Nutrition

University of Kentucky

Lexington, KY 40546-0215

Phone: 859-257-3822

E-Mail: ylxiong@uky.edu

Document #1 - Product Introduction

Document #2 - Capsule / ingredient manufacturing specification

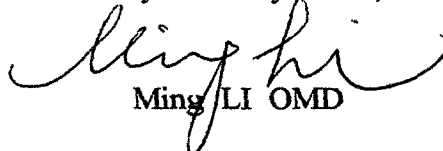
Document #3 - Toxicologic test on food safety

Document #4 - The Immunoregulation Test function

Document #5 - Sarcoma S-180 Suppression Test

I wish to thank you in advance for your attention to this submission.

Very Sincerely Yours,


Ming LI OMD

83889

Q/320217UAL03-2002

Selected Web sites that provide Ganoderma information

- 1) http://www.e2121.com/herb_db/viewherb.php3?viewid=642&setlang=1
- 2) <http://www.hollisny.com/lingzhi.htm> (commercial site)
- 3) <http://www.xianzhilou.com/Chinese/profile.htm> (commercial)
- 4) <http://www.shuanghor.com/> (commercial)
- 5) <http://search.msn.com/results.asp?RS=CHECKED&FORM=MSNH&y=1&q=ling+zhi> (commercial and technical information)
- 6) http://econoherbs.com/reishi/reishi_gano.html (products and sale prices)
- 7) <http://www.mayaka.com/Ganoderma%20Mushroom%20%28LingZhi%29%20And%20Health.html> (information)
- 8) <http://www.wild-lingzhi.com/> (research data included)
- 9) <http://www.hk17.com/ganopoly/English/enet01b.htm> (describes the mechanism of Lingzhi action)

Document translated by:

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University of Kentucky
Lexington, KY 40546-0215
Phone: 859-257-3822
E-mail: ylxiong@uky.edu

Dr. Li:

You may find the above web sites interesting. There are numerous such articles, and I selected just a few for your info. The first one (1) but Natural Health Center is a particularly interesting one.

Yanling

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1. Scope

The standard described hereof stipulates the FBHC product requirement, and its testing methods, analysis, packaging, shipping, and storage. The standard applies to the culture medium comprised of corn flour, glucose, wheat bran, and sodium selenite. It also applies to inoculation of the culture (*Ganoderma lucidum*), fermentation, concentration, and vacuum drying of FBHPC.

2. Literature Used for the Standard

The articles cited in the following documents are used to establish the product standard. Any revisions for the dated citations are not applicable to this standard. However, we encourage the use of the latest versions of the cited documentations in research that is conducted based upon the standard agreed upon. For the undated citations, their latest versions apply to this standard.

GB4789.2~1994	Food Sanitation Microbiological Examinations	Total colony counts
GB4789.3~1994	Food Sanitation Microbiological Examinations	Enterobacteria
GB4789.4~1994	Food Sanitation Microbiological Examinations	<i>Salmonella</i>
GB4789.5~1994	Food Sanitation Microbiological Examinations	<i>Shigella</i>
GB4789.10~1994	Food Sanitation Microbiological Examinations	<i>Staphylococcus aureus</i>
GB4789.11~1994	Food Sanitation Microbiological Examinations	<i>Streptococcus hemolyticus</i>
GB4789.15~1994	Food Sanitation Microbiological Examinations	Mold and yeast counting
GB/T5009.11-1996	Analysis of Total Arsenic in Foods	
GB/T5009.12-1996	Analysis of Lead in Foods	
GB/T5009.17-1996	Analysis of Total Mercury in Foods	
GB/T5009.22-1996	Analysis of Total Aflatoxin B1 in Foods	
GB7718-1991	General Standards for Food Labeling	
GB/T10463-1989	Corn flour	
GB/T12399-1996	Detection of Selenium in Foods	
GB13731-1992	Hard Gelatin Capsule for Drugs	
GB/T14769-1993	Analysis of Food Moisture	
GB16740-1997	General Standards for Health (Functional) Foods	

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JJF1070-2000 Stipulations for Quantity
 Measurement of Quantitatively
 Packaged Products
 National Technology Quantity inspection regulations for
 Inspection Bureau quantitatively packaged
 Ordinance 43, 1995 commercial goods

3. Requirements

3.1 Raw materials

3.1.1. Corn flour

Must meet the GB/T10463 stipulation.

3.1.2. Wheat bran

Must meet the requirements indicated in Appendix A.

3.1.3. Glucose

Must meet the requirements indicated in Appendix B.

3.1.4. Sodium selenite

Must meet the requirements indicated in Appendix C.

3.1.5. *Ganoderma Lucidum*

Classification must conform to microbiology: Basidiomycetes,....

3.1.6. Capsules

Must meet the GB13731 stipulation.

3.2. Sensory indexes

Smooth capsule surface; free of damage, adhesion, and dent; the content inside the capsule should be a light brown powder with a delicate scent and slightly sweet taste characteristic of mushrooms; no off-flavor, impurities, and molding are allowed.

3.3. Functional requirements

The product has the health function of regulating one's immune system.

3.4. Physico-chemical indexes

The capsule content must meet the specifications shown in Table 1.

Table 1. Physico-chemical indexes

Items	Index
Polysaccharides, g/100g	≥ 6.5
Selenium, μg/g	20-40
Moisture content, %	≤10

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Lead (as Pb), mg/kg	≤1.5
Arsenic (as As), mg/kg	≤1.0
Mercury (as Hg), mg/kg	≤0.3
Aflatoxin B1, µg/kg	≤5.0

3.5. Unit net weight

Each unit of the product has a 22.8 g net weight, with an allowed error margin of ±2.0 g. It must accord with the "Stipulations for Quantity Measurement of Quantitatively Packaged Products".

3.6. Microbiological indexes

The indexes must meet the specifications described in Table 2.

Table 2. Microbiological indexes

Items	Index
Total colony count, cfu/g	≤1000
Enterobactria, MPN/100g	≤40
Molds, cfu/g	≤25
Yeasts, cfu/g	≤25
Pathogens (<i>Salmonella</i> , <i>Shigella</i> , <i>Staph. aureus</i> , and <i>S. hemolyticus</i>)	Non-detectable

4. Analytical Methods

4.1. Sensory index

Place 10 g of product sample into a white porcelain plate. Under a good natural light, eyeball the outer surface of the capsule for color, gloss and morphology, and then taste and smell the product.

4.2. Pysico-chemical indexes

4.2.1. Polysaccharides

Analyzed according to Appendix D.

4.2.2. Selenium

Measured according to the GB/T12399 method.

4.2.3. Moisture content

Measured according to the GB/T14769 method.

4.2.4. Lead

Measured according to the GB/T5009, provision 12 method.

4.2.5. Arsenic

Measured according to the GB/T5009, provision 11 method.

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4.2.6. Mercury

Measured according to the GB/T5009, provision 17 method.

4.3.7. Aflatoxin B1

Measured according to the GB/T5009, provision 22 method.

4.3. Net weight of individual products

Measured according to the JJF1070 method.

4.4. Microbiological indexes**4.4.1. Total colony counts**

Determined according to the method stipulated in GB4789 Provision 2.

4.4.2. Enterobacteria

Analyzed according to the method stipulated in GB4789 Provision 3.

4.4.3. Molds and yeasts

Analyzed according to the method stipulated in GB4789 Provision 15.

4.4.4. Pathogens

Analyzed according to the methods stipulated in GB4789.4, GB4789.5, GB4789.10, and GB4789.11.

5. Inspection Rules**5.1. Type of inspections**

Product output inspection; and form inspection

5.1.1. Product output inspection

5.1.1.1 Products that are permitted for release must pass the inspection by the company's quality inspection department.

5.1.1.2. Inspection parameters: sensory perception, polysaccharides, net quantity, total colony counts, and enterobacteria

5.1.2. Form inspection

5.1.2.1. Form inspection is to be conducted when one of the following conditions exists:

- a) Every 6 months during normal production.
- b) Resumption of production after a 3-month or longer period of stoppage.

5.1.2.2. Items to be inspected are those listed in sections 3.2, 3.4, 3.5 and 3.6.

5.2. Batch approval and sampling

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A group of products that are manufactured from the same batch of raw materials, the same work shift, and completely packaged are considered a batch. These products are to be randomly sampled at a rate of 1‰~2‰, with each sample weighing no less than 100 g. Samples for form inspection must be randomly obtained from the pool that had passed the production output inspection.

5.3. Judgment rules

If an inspection item does not meet the standard, the batch from which the product was sampled is allowed to be sampled again for a re-inspection. If the re-inspection fails again, this batch of products can not be certified. Microbiological parameters are ineligible for re-inspection.

6. Label, Packaging, Shipment, and Storage

6.1. Label

6.1.1. The package label must conform to the stipulations of GB7718 and GB16740.

6.1.2. The exterior of the shipment package must clearly show the following: product name, manufacturer's name and address, production date or lot number, the quantity, weight, volume, permission number, certification number, and proper storage/shipment marks that meet the requirements.

6.2. Packaging

The interior packaging material must be an aluminum pouch or a plastic bottle that meet the food sanitation specifications. Each capsule shall weigh 0.38 g, each flat shall contain 12 capsules, or each bottle shall contain 60 capsules. The exterior sale package will be a paper box, each containing 5 flats or 1 bottle. Shipment package will be corrugated paper boxes.

6.3. Shipment

The shipment vehicle must be clean and sanitized. Mixing with poisonous and hazardous substances is strictly prohibited. Care should be taken to avoid contacting with rain, exposing to sun, and absorbing moisture.

6.4. Storage

Products should be placed in a well-ventilated, dry, and cool storage room. No poisonous and hazardous materials shall be stored in the same room.

6.5. Product shelf-life

Under the above shipping and storage conditions, the product will have a shelf-life of 24 months (i.e., quality guaranteed).

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Appendix A

Wheat Bran Quality Index

A.1. Sensory parameters

Powder, a uniformly fresh appearance, free of molding, free of agglomeration, and free of off-odor.

A.2. Impurities

No foreign substances shall be mixed into the wheat bran. No antioxidants, anti-molding agents, or other additives can be added.

A.3. Physico-chemical parameters

Table A1. Physico-chemical indexes

Items	Index
Particle size (passage through mesh 40), %	≥ 99
Moisture, %	≤ 13
Crude protein, g/100g	≥ 13
Crude fiber, g/100g	≤ 10
Crude ash, g/100g	≤ 6

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Appendix B Glucose Quality Index

B.1. Sensory indexes

White crystal powder or white granulated powder, a sweet taste, and free of agglomerates and off-odors.

B.2. Impurities

No sucrose or any other sweetener can be mixed in.

B.3. Physico-chemical indexes

Table B1. Physico-chemical indexes

Items	Index
Glucose, g/100g	≥ 96.0
Specific rotation	$+5.2 \sim +5.3$
Acidity (0.1 mol/NaOH), ml/2.5 g	≤ 0.3
Chlorine compounds (as Cl), mg/kg	≤ 100
Iron compounds (as Fe), mg/kg	≤ 20
Ash, g/100g	≤ 0.2
Moisture, g/100g	< 9.0
Arsenic, mg/kg	≤ 0.5
Lead, mg/kg	≤ 1.0

B. 4. Microbiological indexes

Table B2. Microbiological indexes

Items	Index
Total colony counts, cfu/g	≤ 750
Colon colony counts, MPN/100g	≤ 30
Pathogens (enteric pathogens and pathogenic cocci)	Non-detectable

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Appendix C
Sodium Selenite Quality Index

C.1. Appearance

Colorless crystalline powder.

C.2. Physico-chemical indexes

Table C1. Physico-chemical indexes

Items	Index
Sodium selenite (Na_2SeO_3 , dry weight basis), g/100g	≥ 98.0
Sodium selenite (as Se), g/100g	≥ 44.7
Clarity	clear
Moisture, g/100g	≤ 2.0
Selenate and sulfate	passed

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Appendix D

Analysis of Polysaccharides

D.1. Principle

Polysaccharides are measured by stepwise precipitation using ethanol. When the concentration of added ethanol reaches 30%, proteins, starch, maltodextrin and other large molecules will precipitate, which can be removed through centrifugation. When the alcohol concentration in the supernatant is increased to 70%, glucose and other small molecules will stay soluble, while most polysaccharides will precipitate. Hence, the precipitates may be regarded as polysaccharides.

D.2. Main instruments and reagents

- a) Analytical balance (sensitivity 0.1 mg)
- b) Centrifuge
- c) Homogenizer
- d) 95% ethanol

D.3. Sample preparation

Obtain random samples, thoroughly mix, and then weigh out 5 g.

D.4. Measurement

D.4.1. Suspend the sample (from D.3) in 10 volumes (1:10) of water. After homogenization, incubate the sample solution at 95°C~96°C for 3 h, and then centrifuge at 3500 r.p.m. for 20 minutes. Decant and keep the supernatant.

D.4.2. Treat the precipitate with the same procedure as above (D.4.1) for additional extraction.

D.4.3. Combine the supernatants collected from step D.4.1 and step D.4.2 and measure the total volume. Add ethanol to a 30% (v/v) concentration and stir for 20 minutes. Centrifuge at 3500 r.p.m. for 20 minutes. Collect the supernatant and gradually add, with constant stirring, more ethanol to a 70% (v/v) concentration. Set the solution in a 4°C cooler for approximately 18 hours and then centrifuge at 3500 r.p.m. Wash the pellet with absolute ethanol and dry in a 60°C oven until a constant weight is established. The dried product is polysaccharides

D.5. Calculation

$$\text{Polysaccharides (g/100g)} = \frac{\text{Polysaccharide extracted (g)}}{5 \text{ g}} \times 100$$

440 灵芝 Lingzhi

本品为多孔菌科 (Polyporaceae) 真菌赤芝或紫芝的干燥子实体。

【原植物】

1. 赤芝 *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

子实体有菌柄。菌柄侧生或偏生于菌盖的一侧，近圆柱形，红褐色至紫褐色，具皮壳，硬而有漆样光泽；菌盖，栓质，肾形或半圆形，罕近圆形，宽12~20cm，厚可达2cm；盖面皮壳状，红褐色，具漆样光泽，有同心环纹和辐射状皱纹，边缘平截。菌肉近白色至淡褐色；菌管单层，管口面乳白色，触后变为血红或紫红色，管口宽椭圆形，一端平截，双层壁，内壁有小刺，褐色，外壁光滑，无色。9~12×6~7μm。(图440-1)

2. 紫芝 *Ganoderma sinense* Zhao, Xu et Zhang

子实体有柄。菌盖半圆形，近圆形至近匙形，皮壳坚硬；盖面紫黑色或紫褐色，具漆样光泽，具同心环纹和辐射状菌柄侧生，圆柱形或略扁平，与菌盖同色。菌肉褐色至深褐色。孢子卵形，顶端平截，双层壁，内壁有小刺，外壁平滑。9.5~13.8×6.9~8.7μm。(图440-2)

【生境分布】 生于阔叶树树桩或倒木上。赤芝几乎分布于全国，各地均有栽培；紫芝分布于河北、山东、浙江、江西、福建、台湾、湖南、广东、广西。

【采制】 全年可采，除去泥沙，阴干或晒干。

【化学成分】 赤芝中含有三萜类化合物灵芝酸A、B、C₁、C₂、D₁、D₂、E₁、E₂、F、G、H、I、J、T、U、V、W、X、Y、Z，赤芝酸A、B、C、D、D₁、E₁、E₂、F，灵赤酸A、B、C等，还含有氨基酸、多肽、水溶性蛋白质、酸性蛋白酶、真菌溶菌酶等含氮物质，以及糖类、麦角甾醇、香豆精甙、挥发油、硬脂酸、苯甲酸等，含四种相似灵芝多糖BN₃C₁~BN₃C₄；孢子中含甘露醇、海藻糖和硬脂酸。紫芝含麦角甾醇、有机酸（顺蓖麻酸、延胡索酸等）、氨基葡萄糖、树脂、甘露醇等。

【药理作用】 灵芝能增强巴比妥类药物的中枢抑制作用，具有一定的镇痛作用；具有一定的镇咳、祛痰及平喘作用；灵芝多糖BN₃C能刺激T、B及附属细胞的增生并对抗环磷酰胺抑制小鼠产生脾细胞的作用；灵芝多糖还具有抗肿瘤及增强小鼠网状内皮系统及吞噬功能；家兔口服灵芝浸膏后，能增加血浆皮质醇含量。

【性味功能】 味淡、性温。安神健胃，补益气血。

【主治用法】 用于神经衰弱、失眠、食欲不振、久病体虚及冠心病、高血脂症、慢性气管炎、肝炎、白细胞减少症等。水煎服或入丸服。

【用量】 9~12g。

Ganoderma; Ganoderma

Sources: The drug is the dried fructifications of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. or *G. sinense* Zhao, Xu et Zhang (family *Polyporaceae*), growing on trunks or fallen logs of broad-leaf trees, wild growing or cultivated. *G. lucidum* distributed in almost all parts of China, *G. sinense* distributed in Hebei, Shandong, Zhejiang, Jiangxi, Fujian, Taiwan, Hunan Guangdong, Guangxi.

Constituents: *G. lucidum* contains triterpenes such as ganoderic acid A, B, C₁, C₂, D₁, D₂, E₁, E₂, F, G, H, I, T, U, V, W, X, Y, Z, lucidenic acid A, B, C, D₁, D₂, E₁, E₂, F, ganolucidic acid A, B, C, etc.; amino acids, polypeptide, water-soluble proteins, acidic proteins, fungal lysozyme; also contains saccharides, ergosterol, volatile oils, stearic acid, benzoic acid, 4 kinds of polysaccharides BN₃C₁~BN₃C₄; spores contain mannitol, trehalose, stearic acid; *Ganoderma sinense* contains ergosterol, organic acids such as cis-ricinic acid, fumaric acid etc., aminoglucose, resin, mannitol.

Actions and Indication: It is used as tranquilizer, appetizer, tonic, for the treatment of neurasthenia, insomnia, loss of appetite, weakness due to persistent diseases, coronary heart disease, hyperlipemia, chronic tracheitis, hepatitis, leukopenia, etc.

参考文献

INTRODUCTION

Ganoderma lucidum, so-called lingzhi in China and reishi in Japan, is rare mushroom with medicinal values. In China, lingzhi has been considered to be a panacea tradition for the treatment of diseases. The medicinal values of fungus lingzhi has been described in first Chinese medicinal work 2000 years before, 《Shengnong Ben Cao Jing》, and also in another famous ancient Chinese medicinal book, named 《Ben Cao Gang Mu》.

Ganoderma lucidum is a species of wood-rotting fungi, belonging to the family of polyporaceae. On account of its biological active components, it is reported that *G. lucidum* has been used to treat various human diseases such as: hepatopathy, chronic hepatitis, nephritis, hypertension, hypercholesterolemia, arthritis, neurasthenia, insomnia, amnesia, bronchitis, enteritis, arteriosclerosis, leukemia, diabetes, intoxication and asthenia, especially it becomes a hot medicine to cure tumorigenic diseases. In recent years by artificial solid culture and liquid culture methods, *G. lucidum* products have been produced on large scale and used as clinical drugs, which have the same pharmaceutical effect. It is reported that polysaccharides and Ganoderic acid are the major operative components of *G. lucidum*.

As we know that biological active compounds from *G. lucidum* are obtained by two ways: one is by extraction from the fruiting body of solid cultured *G. lucidum*, the other is by submerged fermentation of *G. lucidum*. The deficiency of solid culture is its longer periodicity, easily influenced by environment condition, and difficult to produce on large industry scale, the latter overcomes the former's shortcomings, it can produce bio-active compound orientationally by optimized controlling the fermentation process, and increase the yield.

The submerged fermentation technology of *G. lucidum* is similar to the fermentation of antibiotic. Research on the submerged fermentation of *G. lucidum* started from 1970's. Currently, many researches focus on obtaining higher biomass. Our procedure makes use of distilled grain, which is a kind of renewable resources and rich in nutrients to be advantageous to the growth of *G. lucidum* and the

production of polysaccharides. It shows an effective pathway for the bioconversion of the renewable resources. Through extraction and purification, product is obtained, which contains bio-active polysaccharide and Ganoderic acid.

Ganoderic acid

It is reported that there are 3 types Ganoderic acids. Type I includes Ganoderic acid A, D, C, K, Land C₂, Type II includes Ganoderic acid Ma, b, c, d, g, h, I, j, Type III includes Ganoderic acid Me, f. Ganoderic acids R, S, T are the major components of submerged fermentation of *G. lucidum*.

Ganoderic acids have effect on protection liver, elimination toxin, reduction cholesterol hypotension and restraint of releasing histamine.

We have researched and tested 3 Ganoderic aids in fermentation products.

Polysaccharides

Polysaccharides from *G. lucidum* are the major components responsible for its biological activities. as we know *G. lucidum* exhibits anti-tumor, activity, liver protective hypoglycemic and platelet aggregation-inhibiting activities. It is reported that polysaccharides with backbone B-1,3-linkages, with suitable molecular size (10×10^5 - 2×10^5), and heteropolysaccharides exhibit higher bio-pharmacological activities.

The polysaccharides produced by our procedure are tested by IR spectrum, ¹³C NMR spectrum, and Gas chromatography (see figure 5-3,5-4,5-7).

The absorption at $3000-2800\text{cm}^{-1}$ in IR spectrum indicate the characteristic absorption of sugar. The characteristic absorption at 891cm^{-1} is indicative of the β -D-glucosidic linkages.

The analysis of figure 5-4:

From 98-110ppm, there are 7 residues conformations:

(1) the chemical shifts of 98.6 ppm and 98.6ppm indicate α -1-2 glycoside linkage or

α -1-2 side chain linkage in polysaccharides

(2) the chemical shifts of 102.5 ppm and 83.5 ppm indicate α -1-4 glycoside linkage in polysaccharides

(3) the chemical shifts of 100.6 ppm and 65.4 ppm indicate α -1-6 glycoside linkage in polysaccharides

(4) the chemical shifts of β -1, 3-glycoside linkage, according with the result of IR spectrum, it is the backbone linkage of the polysaccharide

(5) the chemical shifts of 105.2ppm and 71.5ppm indicate β -1, 6-glycoside linkage in polysaccharides

(6) the chemical shifts of 107.1ppm indicates β -D-arabinose in polysaccharides

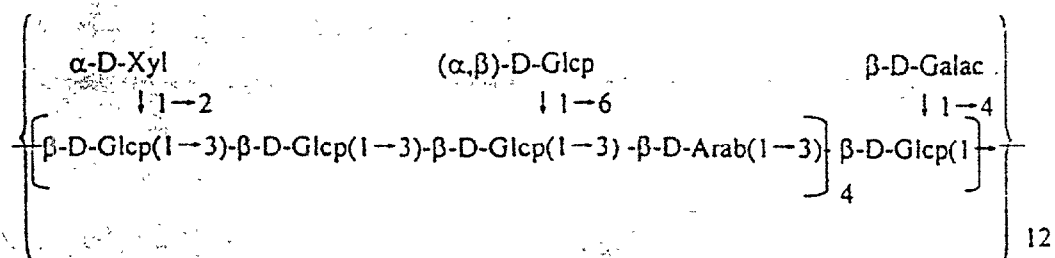
(7) the chemical shifts of 110.2ppm and 80.3ppm indicate β -D-Lactose in polysaccharides

The analysis of Gas chromatography:

(figure 5-5 for standard sample figure 5-6 for polysaccharide sample)

According to the chromatogram, the polysaccharide (IFr1) is composed of glucose, lactose, arabinose and xylose, and their molar ratio is 12:1:4:2

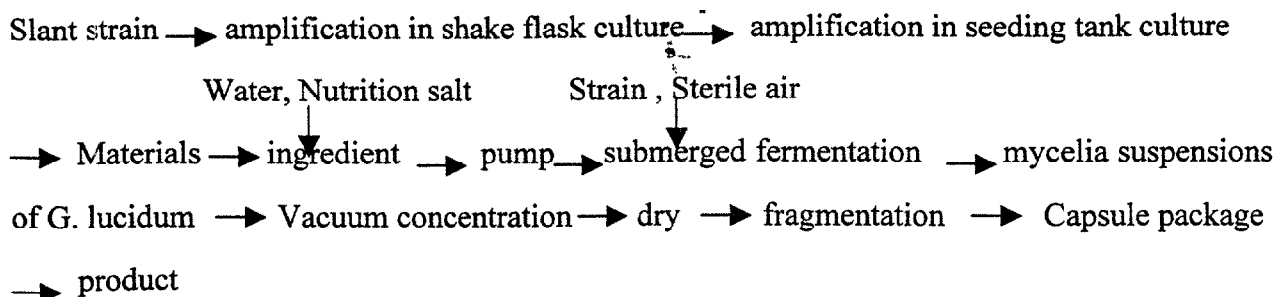
The molecular structure shows below:



Till now, there are many patent products of *G. lucidum*:

- A. anti-tumor products
- B. liver functional improvement products
- C. hypotensive
- D. hypercholestenole drugs
- E. Hypoglycemic
- F. chronic bronchitis drugs
- G. immuno-modulating products
- H. crinks

Jianshenbao capsula product and scheme



Clarification of the product procedure:

1、grain material is main material, and its quality should coincide with the product standard of our company

2、the *Ganoderma lucidum* strain was bought from Jiangsu Microorganism Institute.

The strain was amplified through second-degree shake flash culture and through second-degree seeding tank culture, and then it can be directly used as produce seed.

3、The fermentor is standard ventilatory agitation fermentor, the culture suspension, materials and water are pumped into the fermentor and sterilized by high-pressed steam, after the culture suspension being cool ,the strain seed are inoculated by aseptic manipulation, and culture under ventilation.

4、Fermentation is operated at 28℃

5、Concentration, dry, fragmentation, package are operated in clean condition, fragmentation, package are operated in dry and desiccation condition.

6、The whole procedure, which produces no pollution and toxicants, is a non-pollution process.

Product formula and its basis

The formula's basis

Ganoderma lucidum spore need miscellaneous nutrition substances, such as moisture, carbon sources, nitrogen sources and minerals during its germination, growth and development. Glucose and corn powder provide sufficient carbon sources for Ganoderma lucidum spore during its germination. Corn powder and wheat bran provide necessary nitrogen sources, and the latter contains the indispensable growth factor for Ganoderma lucidum, especially being the prosthetic group of enzyme---the family of vitamin B, phosphate compensates short minerals in moisture and wheat bran to assure the normal life activity of Ganoderma lucidum. The culture medium is obtained through orthogonal test, which suits either for Ganoderma lucidum growth or for high yield of Ganoderma polysaccharides.

By microorganism enriching trace element and bioconverting, sodium selenite are added, which has little effect on the formation of Ganoderma polysaccharides. Se, one the essential trace element for human, is the necessary component of glutathione peroxidase in body to fulfill anti-oxidation and protect cell membrane. Sodium selenite content is obtained according to the recommended allowance of $50\mu\text{g}$ per day for one person. Intake 4 pellets of our product (0.38g/pellet) contains about Se $46\mu\text{g}$.

Studies on Biological Active Compounds Production by Submerged Fermentation of *Ganoderma lucidum*

ABSTRACT

The starting point of this research was to make use of distilled grain which is a kind of renewable resources, and the primary purpose was to produce bioactive polysaccharides and ganoderic acids from submerged fermentation culture by *Ganoderma lucidum*. The following aspects, including fermentation, extraction, isolation, and structure analysis of polysaccharides and ganoderic acids as well, were well studied. Other aspects such as batch fermentation kinetics, rheological properties of pellet suspensions, biologically activity assay of polysaccharides and ganoderic acids were preliminarily investigated.

A correlation between extracellular polysaccharide concentrations and relative viscosity of the culture filtrate was set up, and a fast assay of crude extracellular polysaccharides could be gotten by measuring relative viscosity, yielding an average error was around 4.4%.

The microscopic photograph showed extracellular polysaccharide in the culture of *G. lucidum* which was an adherent polysaccharide. Considering some nutritional factors showed that distilled grain, corn powder, glucose, wheat bran, yeast extract and higher C/N ratio were favorable to the extracellular polysaccharide formation. An optimum medium for industrial scale fermentation was obtained through orthogonal test, which had the following composition(kg/m³): distilled grain(contain 75% water), 80; glucose, 10; corn powder, 10; and wheat bran, 5. The suitable fermentation conditions were determined by non-nutritional factors experiment, which were as follows: initial pH of medium, 5.5; inoculation size (in filamentous morphology), 10%; temperature, 30°C; shaking frequency, 150 r/m; agitation rate, 180 r/m (for 25L fermentor); air velocity, 1:0.75 (v/v/m). The maximum crude extracellular polysaccharides yield was about 2.91kg/m³ (in 25L fermentor) and 2.34 kg/m³ in shaking flask under the above-mentioned medium and conditions. The result of systematic pH controlled during fermentation in a 2L fermentor indicated that the optimum pH of extracellular polysaccharides formation was 4.0, and extracellular polysaccharides yield could be increased to 12% by operating the process at pH4.0.

The change of mycelial morphology during submerged fermentation was studied. The result showed that *G. lucidum* grew in the filamentous form at the early stage (0- 48 h). Pellets were formed after 48 h, and part of filamentous form could be produced after 72h. The size, shape and surface features of pellets varied with environmental conditions. The culture medium, agitation speed, aeration, inoculation size etc. could lead to either loose type or dense type pellets under different medium. The morphology of *G. lucidum* in the submerged fermentation had a considerable effect on production of extracellular polysaccharides in some extent. It was favorable for *G. lucidum* to form filamentous

mycelia, smaller and loose type pellets, this happened when distilled grain, corn powder, glucose, wheat bran and yeast extract were used especially as carbon and nitrogen sources, therefore the yield of polysaccharides was higher than other carbon and nitrogen sources.

The rheological properties of mycelial suspensions of *G. lucidum* were measured, the results indicated that the rheological models of the suspensions at different culture stages had an alternation from Newton model to Bingham model, then to Casson model, and finally to Pseudo-elastic model. Furthermore, influences of biomass, pellets dimension and agitation speed on the rheological behavior were also studied.

Kinetics model of batch fermentation was presented to describe growth of mycelia pellets, substrate consumption and product formation, and had a better simulation values with experimental values, particularly for the pellets growth.

The crude extracellular polysaccharides were precipitated by stepwise addition of ethanol (30% and 60% respectively) at 4°C and pH 4.0. Each precipitate was collected by centrifugation and dried in vacuum. The yields of these precipitates were 0.56 kg/m³, and 2.35 kg/m³ respectively. The intracellular polysaccharides of mycelia produced from submerged fermentation by *G. lucidum* were extracted by the following conditions: extraction temperature, 95°C; extraction time, 3.5h; and mycelia:water ratio; 1:2.5, which was optimized by response surface analysis. The maximum yields of polysaccharides was up to 10.5g/kg dry mycelia, which was the same as the amount in fruit body of *G. lucidum*, then increased 2-folds.

Extracellular polysaccharides were precipitated with 60% ethanol and deproteinized by Sevag's procedure, and protease digestion, this process led to 74.5% of protein removal. The above-treated polysaccharides were applied to a column of DEAE-cellulose (OH⁻ form), and was stepwisely eluted, first with distilled water, second with sodium hydrogen carbonate (0.1mol/l, 0.3mol/l, 0.5mol/l successively) and finally with 0.1mol/l sodium hydroxide to carry out a group separation. Five fractions were obtained, and the main fraction known as fraction I representing 75.6% of total sample amount. Furthermore, fraction I was subjected to chromatography on a column of SepharoseCl-6B, eluted with water at a flow rate of 30ml/h, the relative viscosity of sample solution was kept constant at 1.5. Two fractions, IFr1 and IFr2, were obtained, with a ratio of 4:1, the yield of the two purified fractions was 87.4% of the fraction I. The physics absorption and ion exchange reaction were regarded as the separation principle of DEAE-cellulose ion exchange chromatography for polysaccharides. The main separation parameters of SepharoseCl-6B were also achieved by mathematical calculation and deduction.

Molecular weight of IFr1 and IFr2 estimated by Membrane Osmometer were 38000 and 22000 dalton respectively. The IR spectrum suggested that IFr1 had glycosidic linkages. The ¹³C NMR spectrum of IFr1 indicated that (1) IFr1 was a branched heteropolysaccharide, (2) IFr1 contained a β-(1→3)-linked backbone with side-chains involving α-(1→2), (1→4), (1→6)-glycosidic linkages and β-(1→6)-glycosidic linkages. G C confirmed that IFr1 contained glucose, galactose, arabinose and xylose in the molar

ratio of 12:1:4:2. The molecular structure was also deduced.

Three ganoderic acids in the fermentation broth, named M_1 ($R_f = 0.56$), M_2 ($R_f = 0$) and M_3 ($R_f = 0.17$), were qualified by TLC plates on Silica gel. The TLC was developed with methylbenzene:EtOAc:acetic acid (12:4:0.5) and the spots were detected by the use of methanol:sulfuric acid (1:1, v/v) reagent. A fast UV spectroscopy method for the quantification of crude ganoderic acids in the fermentation broth was also proposed.

The maximum yield of crude ganoderic acids in 25L fermentor was 0.32kg/m^3 . The yield was obtained under the same fermentation medium and conditions as during production of extracellular polysaccharides except that, fermentation period was shortened (80h). In addition, ganoderic acids concentration in the pellets was also assayed, and yield around 0.12kg/kg dry mycelia.

Crude ganoderic acids were applied to chromatography on a column ($30 \times 500\text{mm}$) of Silica gel (C-200) and eluted stepwisely with $\text{CH}_3\text{OH}:\text{CHCl}_3 = 1:9$, $5:95$ and $1:9$ with the ratio of sample:Silica gel of $1:60$, therefore the above-mentioned ganoderic acids were able to be successfully separated. These three compounds had absorption at the range $1200\text{--}1400\text{cm}^{-1}$ of I.R., and maximum absorption at 253nm , 241nm and 223nm with UV spectrum, which could preliminarily lead to a conclusion that M_1 , M_2 and M_3 belong to triterpenoid.

UV spectroscopy and IR spectroscopy indicated that M_1 and M_2 were both belong to tetracyclic triterpenoid acids. ^{13}C NMR spectrum and MS spectrum of M_1 and M_2 confirmed their structures and molecular formula were then $\text{C}_{34}\text{H}_{52}\text{O}_7$ and $\text{C}_{36}\text{H}_{54}\text{O}_7$ respectively, which were the same as reported in the literature.

The animal test showed that; IFr1, crude extracellular polysaccharide and dry powder from fermentation broth could inhibit the growth of Sarcima 180 (solid-type tumor) in mice. The average inhibition ratio was (i.p. 10mg/kg for 10 days) 57.4% (i.p. 10mg/kg for 10 days), 51.2% (i.p. 10mg/kg for 10 days) and 68.0% (v.p. 100mg/kg for 10 days). Immunological activity were also tested, the result showed that IFr1, crude extracellular polysaccharide and dry powder of fermentation broth could significantly improve macrophage swallow ability (cytophagy), and increase the quantity of monocytes ($p < 0.05, 0.01$) via cytoxan in mice. It was found that the anti-tumor and immunological enhancement effect were both proportional to the dosage, particularly, the dry powder of fermentation broth had higher biological activities than other samples, this probably due to other compounds in the sample tested (ganoderic acids).

The anti-microbial test showed that ganoderic acids could inhibit the growth of *Escherichia Coli*, *Aerobacter aerogenus*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Bacillus subtilis*.

Key words: *G. lucidum*, Fermentation, Polysaccharides, Ganoderic acids, Chromatography, Structure analysis, Biological activity, Anti-tumor, Kinetics, Rheological properties

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Nanjing Public Health Prevention Medical Institute

REPORT ON QUALITY TEST

(Food) Test No. 20020021-1

Sample Name Jianshenbao Capsules of Feiling Brand

Sample Submitter Wuxi Feiling Biological SciTech Co. Ltd.

Sample Producer Wuxi Feiling Biological SciTech Co. Ltd.

August 31, 2002

Report of Toxicologic Test on Food Safety

1 Materials And Methods

1.1 Samples The JianShenBao Capsule of Feiling Brand produced by Wuxi Feiling Biotech Co., LTD. The contents of the capsule are brown powder. The recommended uptake dosage for human is 23.3 mg/kg-bw/d (0.7~1.4g/d, 1.4g/d counted; the body weight is estimated 60kg.).

1.2 Experimental Animals KunMing Mice, SD Rats, provided by the Shanghai Lab Animal Center of Chinese Academy of Sciences, License: SCXK (Shanghai) 2002-0010. ICR Mice, provided by the Experimental Animal Center of Nanjing Medical University, License: SCXK (Jiangsu) 2002-0015. License for animal usage: SYXK (Jiangsu) 2002-0057.

1.3 Acute Toxicity Test Forty KunMing Mice, 20 male and 20 female, weights of them ranged from 18 to 22 grams. Horn's Method adopted. The mice were divided into 4 groups according to their body weights randomly, and then were administrated the capsule contents at the dosages of 2150 mg/kg, 4640 mg/kg, 10000 mg/kg and 21500 mg/kg respectively. Each group should include 5 male and 5 female mice. The contents of the JianShenBao Capsule of Feiling Brand were prepared to be a suspension with the 5% amylum solution (cooled after being boiled). After the 14 hours of fasting for food but not for water, the mice were poured the suspension into their stomachs at the volume dosage of 0.4ml/20g-bw in one go. (The pouring operation was divided into two steps in the high dosage groups, with an interval of 3 hours.) The mice were observed for 7 days after the pouring.

1.4 The Mutagenic Test

1.4.1 Ames Test The standard experimental bacterial strains of TA_{97a}, TA₉₈, TA₁₀₀, TA₁₀₂ and the S₉ were all provided by the Office of Toxicology of Shanghai Institute of Treating and Preventing Occupational Diseases. The Standard Petri Dish Method was used. Perform the test with the contents of JianShenBao Capsule of Feiling Brand at the dosages of 10µg/Petri Dish, 40µg/Petri Dish, 200µg/Petri Dish, 1000 µg/Petri Dish, and 5000µg/Petri Dish. Set up a blank control group with distilled water and a positive control group [TA_{97a} (-S₉): atebrian (750µg/Petri Dish); TA₉₈ (-S₉): rubidomycin (6.0µg/Petri Dish); TA₁₀₀ (-S₉) and TA₁₀₂ (-S₉): methylic methane-sulfonic acid esters (1.0µL/Petri Dish); TA_{97a} (+S₉), TA₉₈ (+S₉) and TA₁₀₀ (+S₉): 2- aminofluorene (10.0µg/Petri Dish); TA₁₀₂ (+S₉): 1,8- quinizarin (50.0µg/Petri Dish)].

1.4.2 The Myeloid Cell Micronucleus Test Fifty ICR Mice, 25 male and 25 female, weights of them ranged from 25 to 30 grams. The mice were divided into 5 groups according to their weights randomly, and then were respectively administrated the contents of JianShenBao Capsule of Feiling Brand at the dosages of 1250 mg/kg-bw, 2500 mg/kg-bw, and 5000 mg/kg-bw, whilst the other two groups were administrated the 0.5% amylum solution as the blank control, and the cyclophosphamide (40 mg/kg-bw) as the positive control group. Each group should include 5 male and 5 female mice. The contents of the JianShenBao Capsule of Feiling Brand were prepared to be a suspension with the 5% amylum solution (cooled after being boiled). The mice of each group were poured corresponding solutions into stomachs at the total volume of 0.4 ml/20g(body weight) in a two-step operation with the interval of 24 hours. Then prepared the sternal bone marrow

smears for microscopic examination 6 hours after the second pouring.

1.4.3 The Mice Sperm Aberration Test Twenty-five male KunMing Mice, weighed from 30 to 35 grams, were divided into 5 groups according to the weights randomly, the blank control group (0.5% amylum solution), three testing groups of contents of the JianShenBao Capsule of Feiling Brand at the dosages of 2500 mg/kg·bw/d, 5000 mg/kg·bw/d and 10000 mg/kg·bw/d, and the positive control group (cyclophosphamide, 40 mg/kg·bw/d). Each group contained 5 mice. The contents of the JianShenBao Capsule of Feiling Brand were prepared to be a suspension with the 5% amylum solution (cooled after being boiled). The mice of each group were poured corresponding solutions or suspension into stomachs at the volume of 0.4 ml/20g(body weight) in continuous 5 days, once per day. Then prepared the bilateral epididymis smears on the 35th day after the first pouring.

1.5 Thirty-day Feeding Experiment

1.5.1 Animals and Grouping Eighty delectation SD rats, weighed from 72~100 grams, were divided into 4 groups according to the weights randomly. Each group included 20 rats (10 male and 10 female). Mix the testing product into the feeding stuff. The rats in the blank control group were fed with primary feeding stuff. The three testing groups were low dosage, medium dosage and high dosage group, at the respective dosages of 1.5g, 3.0g, and 6.0g contents of the JianShenBao Capsule of Feiling Brand per 100g feeding stuff. The rats were bred singly in the cage, free for uptake of food and water. Terminate the feeding experiment after 30 days of continuous observation.

1.5.2 Observation Indexes

1.5.2.1 General Condition Observe the behavior and hair color of the rats once per day.

1.5.2.2 Body weight varying situation Weigh the rats once per week.

1.5.2.3 The consumption and utilization rate of feeding stuff Add feeding stuff twice per week, and record the body weights of the rats.

1.5.2.4 Blood Tests Perform the tests with the tail blood on the 30th day of the experiment, including hemoglobin measurement, and determination of the counts of red blood cells, leukocytes, blood platelets, reticulocytes and determination of classification of leukocytes.

1.5.2.5 Blood Biochemical Analysis On the 30th day of the experiment, separate the serum of the blood sampled from the femoral artery. Perform the determinations of Total Protein (TP), Albumin (ALB), alanine transaminase (ALT), aspartate aminotransferase (AST), Glucose, creatinine (CR), urea nitrogen (BUN), uric acid (UA), cholesterol (T-Che) and triglyceride (TG) with the Hitachi-7150 automatic biochemical analyzer.

1.5.2.6 Viscera weighing and histopathological examination After the experiment, kill the rats by depleting the blood from femoral artery. Anatomize the rats at once, to perform the gross examination. Weigh the liver and both kidneys, calculating the viscera/body-weight ratio. Fix the liver, kidneys and upper end of small intestine with formaldehyde solution. Slice up the samples after paraffin embedding. Then perform the histopathological examination after the H.E. staining.

1.5.2.7 Data Statistics Manage the data in each group with the method of the single factor analysis of variance, using the statistical software of EPI 5.

2 Results

2.1 Acute Toxicity Test

Refer to the Table 1 to see the survival situation of the mice in the acute toxicity test of the contents of JianShenBao Capsule of Feiling Brand. The result of the per os LD₅₀ >21.5g/kg·bw was achieved. And no abnormal phenomenon was observed in any system of the mice during the observation period. According to the LD₅₀ grading standard, the product sample tested can be classified as Nontoxic.

Table 1. Survival situation of the mice in the acute toxicity test of the contents of JianShenBao Capsule of Feiling Brand

Dosage (mg/kg)	Mice Number		Number of Survivals	
	Female	Male	Female	Male
2150	5	5	5	5
4640	5	5	5	5
10000	5	5	5	5
21500	5	5	5	5

Instruction: The LD₅₀ that merely indicates the toxic property and intensity of the product tested can not be regarded as the exclusive standard for safety determination, but only as a reference for the further toxicity tests.

2.2 The Mutagenic Test

2.2.1 Ames Test

The results of the Ames test on the contents of the JianShenBao Capsule of Feiling Brand are listed below in Table2-1 and Table2-2. At each test concentration of the product, with or without S₉-mixed liquids added in, the number of the reverted strains, were all under the double folds of the number of the spontaneously reverted strains, whilst, no dose-effect relationship observed. The results in positive control groups indicated the strong mutagenic effect. According to the Ames Criteria, the samples of contents of the JianShenBao Capsule of Feiling Brand we tests are not indicative of positive mutagenic effect.

Table 2-1 Results of the first Ames Test
(average value of 3 dishes ± standard deviation)

Dosage (μg/dish)	TA _{97a}	TA _{97a} + S ₉	TA ₉₈	TA ₉₈ + S ₉	TA ₁₀₀	TA ₁₀₀ + S ₉	TA ₁₀₂	TA ₁₀₂ + S ₉
10	156±11	165±5	44±2	46±2	160±6	166±5	296±6	300±11
40	152±8	158±8	43±3	44±2	153±3	158±4	280±4	287±3
200	147±6	157±3	39±2	41±4	147±6	150±2	274±8	280±10
1000	146±6	147±6	38±3	10±2	142±3	144±2	268±3	273±3
5000	134±6	138±7	35±3	37±2	130±5	142±10	250±2	258±2
Blank Control	146±5	154±6	43±2	44±7	143±9	146±3	271±4	275±5
Positive Control*	2133±135	2647±140	564±25	596±12	2213±96	2619±95	2913±94	3118±103

* positive control group [TA_{97a} (-S₉): atebrian (750μg/Petri Dish); TA₉₈ (-S₉): rubidomycin (6.0μg/Petri Dish); TA₁₀₀ (-S₉) and TA₁₀₂ (-S₉): methylic methane-sulfonic acid esters (1.0μL/Petri Dish); TA_{97a} (+S₉), TA₉₈ (+S₉) and TA₁₀₀ (+S₉): 2- aminofluorene (10.0μg/Petri Dish); TA₁₀₂ (+S₉): 1,8- quinizarin (50.0μg/Petri Dish)]

Table 2-2 Results of the second Ames Test
(average value of 3 dishes \pm standard deviation)

Dosage ($\mu\text{g}/\text{dish}$)	TA _{97a}	TA _{97a} + S ₉	TA ₉₈	TA ₉₈ + S ₉	TA ₁₀₀	TA ₁₀₀ + S ₉	TA ₁₀₂	TA ₁₀₂ + S ₉
10	159 \pm 6	164 \pm 5	46 \pm 2	46 \pm 1	161 \pm 4	167 \pm 6	283 \pm 8	285 \pm 6
40	158 \pm 4	160 \pm 3	44 \pm 3	44 \pm 3	155 \pm 5	158 \pm 4	278 \pm 9	283 \pm 8
200	149 \pm 7	152 \pm 6	41 \pm 2	41 \pm 3	150 \pm 2	153 \pm 6	269 \pm 8	274 \pm 9
1000	146 \pm 6	149 \pm 4	40 \pm 2	41 \pm 3	144 \pm 3	146 \pm 6	254 \pm 6	256 \pm 6
5000	137 \pm 7	139 \pm 4	36 \pm 2	39 \pm 4	130 \pm 6	142 \pm 10	251 \pm 5	257 \pm 5
Blank Control	147 \pm 6	150 \pm 4	43 \pm 3	45 \pm 3	144 \pm 9	147 \pm 4	267 \pm 6	269 \pm 6
Positive Control*	2166 \pm 56	2211 \pm 30	571 \pm 18	605 \pm 18	2237 \pm 70	2644 \pm 97	2721 \pm 154	3039 \pm 57

*positive control group same as the first Ames Test

2.2.2 The Myeloid Cell Micronucleus Test

The data in Table3 show the effect of the contents of the JianShenBao Capsule of Feiling Brand on the micronucleus frequencies of mice myeloid polychromatic erythrocytes (PCE). At each experimental dosage, no significant differences were observed on the micronucleus frequencies of PCE or on the PCE/RBC ratio of the mice between the testing groups and the blank control group. But the results of positive control group indicated the strong mutagenic effect. According to the test results, the samples of contents of the JianShenBao Capsule of Feiling Brand we tests are not indicative of positive mutagenic effect at the testing dosages of 1250 mg/kg-bw, 2500 mg/kg-bw, and 5000 mg/kg-bw.

Table 3. Effect of the contents of the JianShenBao Capsule of Feiling Brand on the mice myeloid cell micronucleus frequency

Sex	Dosage (mg/kg)	Micronucleus			PCE/RBC		
		Number of PCE Observed	Number of PCE with micronucleus	Micronucleus Frequency(‰)	Number of PCE Observed	Number of RBC	PCE/R
Male	1250	5 \times 1000	8	1.6	5 \times 200	1081	0.9
	2500	5 \times 1000	6	1.2	5 \times 200	1075	0.9
	5000	5 \times 1000	7	1.4	5 \times 200	1067	0.9
	Blank Control (0.5%amylum)	5 \times 1000	12	2.4	5 \times 200	1099	0.9
	Positive Control(CTX)	5 \times 1000	232	46.4	5 \times 200	1301	0.7
Female	1250	5 \times 1000	8	1.6	5 \times 200	1087	0.9
	2500	5 \times 1000	6	1.2	5 \times 200	1085	0.9
	5000	5 \times 1000	7	1.4	5 \times 200	1061	0.9
	Blank Control (0.5%amylum)	5 \times 1000	8	1.6	5 \times 200	1103	0.9
	Positive Control(CTX)	5 \times 1000	204	40.8	5 \times 200	1281	0.7

2.2.2 The Mice Sperm Aberration Test

The data in Table 4 show the effect of the contents of the JianShenBao Capsule of Feiling Brand on the mice sperm aberration rate. The sperm aberration rate in blank control group was 2.42%, which is in the normal range of mice sperm aberration rate (0.87~3.4%). The sperm aberration rate in positive control group was 10.40%, which indicated a strong mutagenic effect.

The sperm aberration rates in the three sample-testing groups were 2.24%, 2.22% and 2.20%, respectively, which showed no significant difference from that in blank control group (2.42%). The samples of contents of the JianShenBao Capsule of Feiling Brand we tests are not indicative of positive mutagenic effect on mice sperm.

Table 4. The effect of the contents of the JianShenBao Capsule of Feiling Brand on the mice sperm aberration rate

Groups	Dosage(mg/kg.bw/d)	Mice Number	Number of sperms tested	Number of aberrated sperms	Aberration Rate
The Capsule contents	2500	5	5000	112	2.24
	5000	5	5000	111	2.22
	10000	5	5000	110	2.20
Blank Control (0.5%amylum)		5	5000	121	2.42
Positive Control(CTX)	40	5	5000	520	10.40

2.3 Thirty-day Feeding Experiment

2.3.1 General condition During the whole experiment, hair color of the rats were normal, no abnormal behavior or death was observed.

2.3.2 Weight situation of the rats in the experiment Refer to Table 5. The body weights of all male and female rats in each testing group showed no significant difference from that of the same sexual rats in blank control group.

Table 5. The effect of a 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand on the rats body weight (average value \pm standard deviation, n=10)

Groups	0 week	1 week	2 weeks	3 weeks	4 weeks
♀Blank Control	84.7 \pm 7.5	128.6 \pm 8.0	151.9 \pm 7.6	168.4 \pm 7.0	182.1 \pm 9.3
♀Low Dosage	89.0 \pm 8.9	134.6 \pm 7.3	157.0 \pm 9.3	178.1 \pm 10.6	197.1 \pm 14.2
♀Medium Dosage	85.1 \pm 8.3	131.4 \pm 8.6	152.3 \pm 11.3	171.2 \pm 10.6	190.0 \pm 12.8
♀High Dosage	84.1 \pm 8.1	127.1 \pm 5.5	152.3 \pm 10.8	170.6 \pm 15.4	187.4 \pm 15.4
♂Blank Control	85.9 \pm 8.9	132.5 \pm 8.2	188.0 \pm 11.7	234.3 \pm 11.7	278.8 \pm 11.5
♂Low Dosage	87.2 \pm 7.7	136.2 \pm 8.3	189.7 \pm 12.5	238.2 \pm 17.5	287.8 \pm 21.2
♂Medium Dosage	85.5 \pm 7.4	133.8 \pm 7.3	185.9 \pm 11.2	230.9 \pm 14.9	275.2 \pm 14.4
♂High Dosage	85.0 \pm 8.0	132.0 \pm 8.6	186.8 \pm 11.0	232.3 \pm 10.7	280.4 \pm 13.2

2.3.3 Food utilization rate The food utilization rate is calculated by the fraction formula: the increase of rat's body weight (g)/100g (feeding stuff). The experiment results are shown in the Table 6. The single factor analysis of variance indicated that the food utilization rates of all male and female rats in each testing group were observed no significant difference from that of the same sexual rats in blank control group.

Table 6. The effect of a 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand on the rats food utilization rates

Groups	Increase of body weight (g)	Food uptake (g)	Food utilization rate (%)
♀Blank Control	97.4±10.9	422.5±24.7	23.0±2.1
♀Low Dosage	108.1±14.7	453.0±28.7	23.9±3.1
♀Medium Dosage	104.9±17.4	415.3±44.7	25.3±3.1
♀High Dosage	103.3±18.7	426.7±45.3	24.1±2.2
♂Blank Control	192.9±13.8	548.8±28.5	35.2±2.4
♂Low Dosage	200.6±19.2	573.3±40.6	35.0±2.7
♂Medium Dosage	189.7±16.4	547.7±20.9	34.6±2.6
♂High Dosage	194.5±14.1	580.5±36.7	33.6±3.4

2.3.4 Blood tests The value of hemoglobin, red blood cells, leukocytes, blood platelets, and reticulocytes determined in the end stage of the experiment are shown in the Table 7 and Table 8. No obvious difference was observed among the testing groups and the blank control group.

Table 7. Blood test results of the 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand

Groups	Hemoglobin (g/L)	RBC ($10^{12}/L$)	WBC ($10^9/L$)	Blood platelet ($10^9/L$)	Reticulocyte ($10^9/L$)
♀Blank Control	139.2±9.0	7.06±0.32	12.6±3.11	967.4±113.0	12.9±1.16
♀Low Dosage	139.7±11.1	7.25±0.49	10.4±3.35	902.5±256.2	13.8±1.63
♀Medium Dosage	143.3±5.0	7.28±0.31	11.8±3.21	959.6±99.4	14.1±1.54
♀High Dosage	143.8±9.2	7.34±0.63	10.2±2.79	824.7±158.8	13.9±1.87
♂Blank Control	139.3±8.3	7.28±0.47	13.1±2.06	944.3±116.6	14.1±2.05
♂Low Dosage	145.5±7.5	7.51±0.45	11.6±3.18	952.6±153.6	13.6±1.74
♂Medium Dosage	144.8±5.8	7.39±0.31	11.2±3.67	878.8±108.6	13.4±1.58
♂High Dosage	144.3±7.1	7.47±0.42	11.5±2.34	741.1±232.7	14.0±1.33

2.3.5 Blood biochemical analysis The value of serum total protein (TP), albumin (ALB), alanine transaminase (ALT), aspartate aminotransferase (AST), glucose, creatinine (CR), urea nitrogen (BUN), uric acid (UA), cholesterol (T-Che) and triglyceride (TG) of the rats in each group were all in normal range. See Table 9-1 and Table 9-2.

Table 8. The classification of leukocytes after the 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand

Groups	Number of rats	Neutrophilic granulocyte (%)	Basophilic granulocyte (%)	Eosinophils (%)	Lymphocyte (%)	Monocyte (%)
♀Blank Control	10	0.60	0.61	67.90	0.41	30.48
♀Low Dosage	10	0.54	0.62	70.79	0.41	27.64
♀Medium Dosage	10	0.57	0.70	71.01	0.47	27.25
♀High Dosage	10	0.48	0.60	72.98	0.53	25.41
♂Blank Control	10	0.68	0.59	70.02	0.38	28.33
♂Low Dosage	10	0.56	0.65	73.31	0.62	24.86
♂Medium Dosage	10	0.56	0.69	71.99	0.64	26.12
♂High Dosage	10	0.43	0.62	69.04	0.53	29.38

Table 9-1. The serum biochemical analysis results of the rats after the 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand (A) (average value \pm standard deviation, n=10)

Groups	TP (g/L)	ALB (g/L)	ALT (U/L)	AST(U/L)	GLU (mmol/L)
♀Blank Control	79.0 \pm 2.5	40.5 \pm 1.7	48.7 \pm 5.8	150.4 \pm 55.8	4.92 \pm 0.82
♀Low Dosage	78.8 \pm 0.9	39.8 \pm 1.0	50.5 \pm 7.1	103.9 \pm 23.5	5.17 \pm 0.59
♀Medium Dosage	77.1 \pm 1.9	40.4 \pm 1.4	53.2 \pm 13.9	112.4 \pm 44.2	4.88 \pm 0.40
♀High Dosage	75.8 \pm 2.0	40.9 \pm 1.8	51.4 \pm 14.9	113.2 \pm 43.5	4.85 \pm 0.41
♂Blank Control	76.5 \pm 2.6	38.5 \pm 2.0	40.6 \pm 3.4	178.3 \pm 68.3	4.08 \pm 0.58
♂Low Dosage	78.0 \pm 1.4	39.6 \pm 1.1	49.4 \pm 10.1	103.4 \pm 22.6	4.62 \pm 0.63
♂Medium Dosage	76.0 \pm 2.1	38.2 \pm 1.2	49.3 \pm 14.3	113.6 \pm 43.6	5.10 \pm 0.45
♂High Dosage	75.7 \pm 2.6	39.4 \pm 1.8	55.3 \pm 18.0	102.7 \pm 27.5	5.03 \pm 0.53

Table 9-2. The serum biochemical analysis results of the rats after the 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand (B) (average value \pm standard deviation, n=10)

Groups	CR (μ mol/L)	BUN (mmol/L)	UA (μ mol/L)	T-Che (mmol/L)	TG (mmol/L)
♀Blank Control	96.7 \pm 16.5	7.34 \pm 1.04	115.9 \pm 52.8	2.09 \pm 0.25	1.32 \pm 0.34
♀Low Dosage	100.6 \pm 7.6	6.48 \pm 0.42	109.2 \pm 25.0	1.97 \pm 0.15	1.39 \pm 0.37
♀Medium Dosage	102.4 \pm 5.2	5.90 \pm 0.44	82.9 \pm 25.4	1.88 \pm 0.40	1.26 \pm 0.19
♀High Dosage	103.1 \pm 3.7	6.02 \pm 0.61	104.6 \pm 31.9	1.50 \pm 0.23	1.39 \pm 0.32
♂Blank Control	86.3 \pm 15.3	6.67 \pm 0.52	94.3 \pm 22.3	1.93 \pm 0.21	1.35 \pm 0.15
♂Low Dosage	101.2 \pm 10.5	6.44 \pm 0.51	107.3 \pm 23.1	1.78 \pm 0.34	1.27 \pm 0.25
♂Medium Dosage	102.2 \pm 3.7	5.50 \pm 0.74	97.3 \pm 23.5	1.63 \pm 0.35	1.39 \pm 0.33
♂High Dosage	104.0 \pm 5.6	5.93 \pm 0.61	87.1 \pm 31.2	1.55 \pm 0.21	1.29 \pm 0.29

2.3.6 Viscera/body weight ratio The data in Table 10 show the effect of the contents of the JianShenBao Capsule of Feiling Brand on the rat viscera/body weight ratio.

Table 10. The effect of a 30-day feeding experiment with the contents of the JianShenBao Capsule of Feiling Brand on the rat viscera/body weight ratio

Groups	Liver/body weight (%)	Kidney/body weight (%)
♀Blank Control	3.85 \pm 0.44	0.75 \pm 0.04
♀Low Dosage	3.62 \pm 0.29	0.73 \pm 0.04
♀Medium Dosage	3.71 \pm 0.66	0.74 \pm 0.10
♀High Dosage	3.83 \pm 0.33	0.75 \pm 0.03
♂Blank Control	3.79 \pm 0.30	0.77 \pm 0.06
♂Low Dosage	4.03 \pm 0.27	0.70 \pm 0.03
♂Medium Dosage	4.09 \pm 0.21	0.72 \pm 0.01
♂High Dosage	4.03 \pm 0.41	0.73 \pm 0.03

2.3.7 Histopathological examination In the anatomizing operation, the gross appearances of the livers, kidneys and duodenums in situ in all product-testing groups of low, medium and high dosages and control groups showed no significant abnormalities. No pathological variations was found in the microscopic observation of the histologic sections of the livers, kidneys and duodenums of the rats in blank control group and high dosage testing group. Microscopic findings of livers, kidneys and duodenums were: the funicular arrangement of the hepatocytes, no degeneration or necrosis, no enlargement of sinus hepaticus, intact structure of hepatic lobules, no infiltration of inflammatory cells or hyperplasia of fibrous and connective tissue in portal area, no degeneration or necrosis of renal tubule epithelium, no renal tubule cast, no hyperemia or edema or inflammatory infiltration in renal mesenchyme, clear structure of each layer of the duodenal wall, intact mucosa epithelium of duodenum, no atrophy, hyperplasia or metaplastic of the submucosa glands, no hyperemia or dilation of the blood vessels in interstitial connective tissue, no obvious inflammatory infiltration, intact

structure of the duodenal muscular layer, no inflammatory exudation of the duodenal mucosa. In comparison with the pathological findings in the control groups, no histopathological changes due to the product samples tested was observed.

2.3.8 Result analysis of the 30-day feeding experiment

No abnormal findings of the general condition, body weight, increase of body weight, food utilization rate, blood regular tests, blood biochemical tests, and viscera weights in the testing groups of all three dosages and the histopathological examinations in the high dosage group were found. Under the conditions of this experiment, that is, at the dosages of 6.31 g/kg.bw/d for male rats and 5.89 g/kg.bw/d for female rats in high dosage group which are respectively 270 and 252 folds of the recommended human uptake dosage (0.0233 g/kg.bw/d), no anomaly was observed after the 30-day continuous feeding with the contents of the JianShenBao Capsule of Feiling Brand. The data of uptake of the contents of JianShenBao Capsule of Feiling Brand in the high dosage group are listed in Table 11.

Table 11. The uptake of the contents of JianShenBao Capsule of Feiling Brand in the high dosage group

Sex	Body Weight* (g)	Uptake amount of feeding stuff (g/d)	Uptake amount of the capsule contents (g/d)	Dosage (g/kg.bw/d)	Folds of recommended human uptake dosage
Femal	144.30	14.22	0.85	5.89	252
Male	183.88	19.35	1.16	6.31	270

*The average value of the body weights of every week

3 Conclusion

3.1 The LD₅₀ value (per os, of mice) of the contents of the JianShenBao Capsule of Feiling Brand produced by Wuxi Feiling Biotech Co., LTD. is above 21.5g/kg.bw, which indicate that the product sample is Nontoxic, according to the Acute Toxicity (LD₅₀) Grading Standards.

3.2 The negative results of the mutagenic toxicity test (Ames Test), myeloid micronucleus test, and mice sperm aberration test indicate non-mutagenic effect of the contents sample of the JianShenBao Capsule of Feiling Brand.

3.3 The results of the 30-day feeding experiment of the SD rats indicate that no abnormal findings of the general condition, body weight, increase of body weight, food utilization rate, blood regular tests, blood biochemical tests, and viscera weights in the testing groups of all three dosages and the histopathological examinations in the high dosage

(96) No. liangren(su)zi (Z0311)

The School of Public Health, Southeast University
Nanjing Public Health Prevention Medical Institute

REPORT ON QUALITY TEST

(Food) No. Jianzi 20020021-2

Sample Name JianShenBao Capsule of FeiLing Brand

Sample Submitter Wuxi Feiling Biological SciTech Co. Ltd.

Sample Producer Wuxi Feiling Biological SciTech Co. Ltd.

August 31, 2002

Nanjing Public Health Prevention Medical Institute Report on Health Food Function Test

(Food) No. Jianzi 20020021-2

P2 of 7 Pages

Sample Name JianShenBao Capsule of FeiLing Brand **Test Type** consign
Sample Submitter Wuxi Feiling Biological SciTech Co. Ltd. **Tel** (0510)2709489
Submitter Add. No. 118 Jiankang Road, Wuxi City **Zip** 214028
Sampling Place No. 118 Jiankang Road, Wuxi City **Submission Date** 2002/07/01
Batch/Date of leaving factory 20010717 **Sample Character** the contents are brown powder
Submission Operator Mao Jian **Sample Quantity** 0.35g/capsule × 12capsules/board × 5boards/box × 100boxes

PURPOSE & PROGRAMS OF THE TEST

PURPOSE: Test the immunoregulation function of this product according to the Immunoregulation Function Test Method in the < Health Food Function Evaluation Program & Test Method > issued by The Ministry of Health.

PROGRAMS:

1. Body weight, ratio of spleen weight / body weight, ratio of thymus weight / body weight.
2. The test of ConA induced spleen lymphocyte conversion in mice (MTT method)
3. Dinitrofluorobenzene (DNFB) induced lagged allergy in mice (ear tumefaction method)
4. Antibody genesis cell detection (Jerne improved glassslide method)
5. Serum hemolysin determination (blood coagulation method)
6. Carbon clearance test in mice
7. The mice abdominal cavity macrophage phagocytizing cock red blood cell test. (half in vivo method)
8. NK cytoactive determination [lactate dehydrogenase (LDH) determination method]

TEST RESULT & JUDGEMENT

1. After the oral injection in the mice with the contents of the JianShenBao Capsule of FeiLing Brand, at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body), no adverse effect on body weight, the ratio of spleen weight / body weight, the ratio of thymus weight / body weight of mice has been found out.
2. The test of ConA induced spleen lymphocyte conversion in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice lymphocyte multiplication capacity at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
3. The result of the dinitrofluorobenzene (DNFB) induced lagged allergy test in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the action of the mice T-lymphocyte multiplying to sensitized lymphocyte at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body)
4. The test result of the antibody genesis cell detection has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can increase the number of the mice antibody genesis cell at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
5. The test result of the serum hemolysin determination has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can promote the generation of mice antibody at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).

6. The result of the carbon clearance test in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the carbon clearance ability of mice at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
7. The result of the mice abdominal cavity macrophage phagocytizing cock red blood cell test has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the phagocytosis ability of mice abdominal cavity macrophage at the dosages of 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 10, 20 times of the commended dosage used for human body).
8. The test result of the NK cytoactive determination has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice NK cytoactive at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).

According to the result judgement principle of the Immunoregulation Function Test Method in the < Health Food Function Evaluation Program & Test Method >, the JianShenBao Capsule of FeiLing Brand manufactured by Wuxi Feiling Biological SciTech Co. Ltd. has the immunoregulation function.

Compiler_____

Reviser_____

Chief of the Lab_____

Seal of the test institution

Auditor_____

Signed & Issued by_____

August 31, 2002

Test Report of the Immunoregulation Function of the JianShenBao Capsule of FeiLing Brand

1. MATERIALS & METHOD

1.1 SAMPLE: the JianShenBao Capsule of FeiLing Brand manufactured by Wuxi Feiling Biological SciTech Co. Ltd. The contents are brown powder. The commended dosage used for human body is 11.7mg/kg, bw/d (0.7~1.4g/d, calculated as 0.7g/d ; the human body weight is calculated as 60kg).

1.2 EXPERIMENT ANIMALS & CELLS: Kunming mice, BALB/C mice, body weight 18~22g, supplied by Shanghai Experiment Animal Center of Chinese Academy of Sciences (IMCAS), license number: SCXK (hu) 2002-0010; Guineapigs, supplied by Jiangning Qinglongshan Animal Culture Farm, license number: SCXK (su) 2002-0004, animal using license number: SYXK (su) 2002-0057. YAC-1 cells, supplied by Microbiology Staff Room in Medical College of Southeast University. The mice are randomly to one normal control group (i.g. 0.5% starch solution) and three groups oral injected with the contents of the JianShenBao Capsule of FeiLing Brand at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage as 11.7mg/kg used for human body). The suspension of the contents of the JianShenBao Capsule of FeiLing Brand is prepared with 0.5% starch solution (boiled, and then cool down). All experiment animals are supplied with full-price granular feed, and can drink water freely.

1.3 METHOD: Make the experiments according to the Immunoregulation Function Test Method in the < Health Food Function Evaluation Program & Test Method > issued by The Ministry of Health. The volume of the solution for oral injection is 0.4ml/20g (in compliance with the tested animals' weight), bw, once per day, 28d's duration.

1.3.1 The test of ConA induced spleen lymphocyte conversion in mice (MTT method).

After the sterile filtration of PRMI1640 culture solution with millipore filter, 10% calf serum, 1% glutamine (200mmol/l), penicillin (100U/ml), streptomycin (100µg/l), and 2-mercaptoethanol (5µmol/l) are added. Use the sterile HCL (1mol/l) or NaOH (1mol/l) to adjust the pH to 7.0~7.2 to make the full culture solution. Make MTT solution (5mg/ml) with PBS buffer solution (pH=7.2~7.4) just before the use. Use the sterile NaOH (3.5%) to adjust the sterile Hanks solution's pH to 7.2~7.4. After the sterile filtration, the ConA solution (100µg/ml) made with twice-distilled water is preserved at the temperature of -20°C. 4ml HCL (1mol/l) is added into the 96ml isopropanol to make the acidic isopropanol solution. The animals (female BALB/C mice, 10mice per group) in each group should be oral injected continuously until the 28th day, and then every mouse is killed by neck-bone-disjoint, the spleen is taken out with asepsis, and then the individual cell suspension is prepared after the lacerate of the spleen with forceps in a small plate dish containing q.s. sterile Hanks solution. This individual cell suspension are filtrated with 200-spore filter, washed with Hanks solution time after time, suspending in 2ml full culture solution after centrifugation. And then count living cells with a phenolphthalein-staining meter, adjust the cell concentration to 2×10^6 cells/ml, add the suspension into 2 culture holes separately, 1ml per pore, add 50µg/l ConA solution into one hole, make the other one for comparison, and then culture them in 5% CO₂ at 37°C for 72h, when 4h before the end of the culture, lightly suck 0.7ml supernatant out from each hole, then add 0.7ml PRMI1640 culture solution (without calf serum) into both holes separately, add 50µl MTT solution at the same time, go on to culture for 4h. When the culture is complete, add 1ml acidic isopropanol solution into both holes separately, blow and stir to uniform the mixture, dissolve the violet crystal thoroughly, and transfer each solution

into 1ml cuvette, determine the optical density at 570nm with 721-spectrophotometer.

1.3.2 Dinitrofluorobenzene (DNFB) induced lagged allergy in mice (ear tumefaction method)

Weigh DNFB 50mg and put it into a dry clean small bottle, further add 5ml acetone-oleumsesami solution (already prepared, acetone/oleumsesami=1/1), tightly plug and seal the bottle with adhesive plaster, stir to uniform the contents, then the DNFB solution has been prepared (prepare the solution just before use, and suck the solution through the bottle-cover with a 250 μ l injection when use.). The animals (male Kunming mice, 10mice per group) in each group should be oral injected continuously until the 23th day, dehair abdominal fur (within an area as about 3cm \times 3cm) of every mouse with BaS, sensitize the area with equably painting 50 μ l DNFB solution. 5d later (no halt of oral injection during this period), attack every mouse with equably painting 10 μ l DNFB solution on both sides of its right ear, 24h after the attack, every mouse is killed by neck-bone-disjoint, obtain two pieces of ear-parts (each diameter = 8mm.) separately from both left & right ears of every mouse with pin-hole plotter, and then weigh them.

1.3.3 Antibody genesis cell detection (Jerne improved glassslide method)

Use the blood of healthy sheep, after defibrination and times of NS wash, to prepare 2%(v/v) sheep red blood cell suspension with NS. Sample blood from 5 healthy guineapigs, abstract the serum and mix it, and then add 5ml guineapig serum into each 1ml press-accumulated sheep red blood suspension (prepared after defibrination and times of wash), set in a refrigerator at 4 $^{\circ}$ C for 30min, shake it frequently, and then prepare alexin (dilute it with barbital buffer solution according to the ratio as 1/10 when use) with supernatant which sucked after centrifugation. The animals (male Kunming mice, 10mice per group) in each group should be oral injected continuously until the 23th day, and then every mouse is intraperitoneal injected with 0.2ml of 2%(v/v) sheep red blood cell suspension. 5d later (no halt of oral injection during this period), every mouse is killed by neck-bone-disjoint, the spleen is taken out with asepsis, and then the individual cell suspension is prepared in a small plate dish containing q.s. sterile Hanks solution. This individual cell suspension are filtrated with 200-spore filter, washed with Hanks solution time after time, suspending in 5ml PRM11640 culture solution. And then count the cells, adjust the cell concentration to 5×10^6 cells/ml. Heat and thaw the surface layer of the culture medium (1g agar added with twice-distilled water to 100ml), preserve heat in water bath at 45 $^{\circ}$ C, and then mix it with equal volume of Hanks solution (of twi-concentration, and pH 7.2~7.4), after that, fractional pack the mixture with small tubes, 0.5ml per tube, and then add 50 μ l of 10% sheep red cell suspension (v/v, prepared with barbital buffer solution) and 20 μ l of spleen cell suspension into each tube, mix the solution thoroughly and rapidly, pour it onto the glass slice on which agarose film has already been brushed. After the solidification of agar, set the slice on the rack horizontally, incubate in CO₂ culture chamber for 1.5h, and then add the diluted alexin into the fluting of the slice-rack, continue to warm and incubate for another 1.5h, after that, count the void spots.

1.3.4 Serum hemolysin determination (blood coagulation method)

Use the blood of healthy sheep, after defibrination and times of NS wash, to prepare 2%, 0.5%(v/v) sheep red blood cell suspension with NS. The animals (male Kunming mice, 10mice per group) in each group should be oral injected continuously until the 23th day, and then every mouse is intraperitoneal injected with 0.2ml of 2%(v/v) sheep red blood cell suspension. 5d later (no halt of oral injection during this period), suck the blood and inject it into the centrifuge tube after the eyeball extirpation on the mouse, standing for 1h, then centrifuge for 10min at speed of 2000r/min, abstract and collect the serum, the multiple proportion dilution of the serum is done with NS in the

micro blood coagulation test board, 100 μ l per hole, further add 100 μ l of 0.5%(v/v) sheep red blood suspension, mix thoroughly, place the micro blood coagulation test board into a moist plate dish and cover it, after 3h incubation in the culture chamber at 37 $^{\circ}$ C, observe the agglutination degree of the red blood.

1.3.5 Carbon clearance test in mice

Use the India ink to prepare the injection-ink after a quadruplication dilution with NS. Weigh 1.0g Na₂CO₃ and add distilled water to a 1000ml Na₂CO₃ solution. The animals (male Kunming mice, 10mice per group) in each group should be oral injected continuously until the 28th day, and then inject injection-ink (0.1ml/10g, bw) into the tail vein of mouse, immediately time after the injection, sample 20 μ l of blood from the angular vein separately at the time points of 2min, 10min after the injection, add the blood into 2ml Na₂CO₃ solution, determine the optical density with 721-spectrophotometer at the wavelength of 600nm, use the Na₂CO₃ solution as blank control. Kill the by neck-bone-disjoint, take out the liver and spleen, suck off the bloodiness from the viscera surface, and then weigh them.

1.3.6 The mice abdominal cavity macrophage phagocytizing cock red blood cell test (half in vivo method).

Use the blood of healthy cock, after defibrillation and times of NS wash, to prepare 20%(v/v) cock red blood cell suspension with NS. The animals (male Kunming mice, 10mice per group) in each group should be oral injected continuously until the 28th day, and then every mouse is intraperitoneal injected with 1ml of 20%(v/v) cock red blood cell suspension. 30min later, kill the mouse by neck-bone-disjoint, fasten the mouse on the mouse-board with supine position, scissor the skin in the middle of sbdominal wall, inject 2ml NS into the abdominal cavity, roll the mouse-board for 1min, suck out 1ml of lotion from the abdominal cavity, drop the lotion onto 2 glassslides separately, set the slides into a enamel box padded with wet gauze, and then move the box into a 37 $^{\circ}$ C culture chamber for a 30min's incubation. After that, take it out, rinse and clean out the no picking cells. After air-drying, fixation with acetone-methanol solution (acetone / methanol = 1 / 1), followed by a 3min staining with 4%(v/v) Giemsa-H₃PO₄ buffer solution, and then rinse it with distilled water, air-drying, observe with an optical microscope at the end.

1.3.7 NK cytoactive determination [lactate dehydrogenase (LDH) determination method]

Subculture the target cell (YAC-1 cell) for 24h just before the test, and then wash with Hanks solution for 3 times before use, and then adjust the cell concentration to 1×10^6 cells/ml. The animals (male BALB/C mice, 10mice per group) in each group should be oral injected continuously until the 28th day, and then every mouse is killed by neck-bone-disjoint, the spleen is taken out with asepsis, and then the individual cell suspension is prepared in a small plate dish containing q.s. sterile Hanks solution. This individual cell suspension are filtrated with 200-spore filter, washed with Hanks solution time after time, suspending in 2ml of RPMI1640 full culture solution. And then count cells, adjust the cell concentration to 5×10^6 cells/ml. Obtain 100 μ l separately from the suspension of target cells and spleen cells, and then add the samples into a 96-hole culture board of U-type, add 100 μ l of target cell suspension and 100 μ l of culture solution into the target cell natural release hole, add 100 μ l of target cell suspension and 100 μ l 1%NP40 into the target cell maximum release hole. Each of the above items are set with three repetition holes, culture them in 5% CO₂ at 37 $^{\circ}$ C for 4h, and then centrifugate that 96-hole culture board at the speed of 1500r/min for 5min, lightly suck 100 μ l supernatant out from every hole and transfer it into a flat bottom 96-hole culture board, at the same time, add 100 μ l LDH substrate solution,

after a 3min reaction, add 30 μ l of 1mol/l HCl, determine the optical density at 490nm with enzyme calibrating meter.

1.3.8 Data statistics

The data obtained from the experiments are treated with the single factor variance analysis with SAS6.12 statistics software for the comparison among the experimental groups.

2. RESULT

2.1 Body weight, ratio of spleen weight / body weight, ratio of thymus weight / body weight

The result of the JianShenBao Capsule of FeiLing Brand contents' effect on the body weight, the ratio of spleen weight / body weight, the ratio of thymus weight / body weight of mice is given out in Tab1-1 ~ Tab1-7 and Tab2. The result have indicated, compared with the normal control group, no significant difference ($P>0.05$) of the body weight, the ratio of spleen weight / body weight, the ratio of thymus weight / body weight of the mice has been found out in each dosage group with oral injection of the JianShenBao Capsule of FeiLing Brand contents.

Tab1-1 the body weight (BW) & body weight increase of mice in spleen lymphocyte conversion test. (mean \pm SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	20.0 \pm 1.0	23.5 \pm 0.5	26.8 \pm 1.3	6.8 \pm 1.7
58.5	10	20.0 \pm 0.8	23.8 \pm 0.8	26.4 \pm 1.0	6.5 \pm 1.2
117.0	10	20.0 \pm 0.9	23.9 \pm 0.8	25.8 \pm 1.3	5.8 \pm 1.7
234.0	10	20.0 \pm 0.8	23.9 \pm 1.0	26.3 \pm 1.2	6.4 \pm 1.7

Tab1-2 the BW & BW increase of mice in lagged allergy test. (mean \pm SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	21.3 \pm 0.5	32.4 \pm 2.5	42.3 \pm 2.1	20.1 \pm 1.9
58.5	10	20.8 \pm 0.9	32.0 \pm 3.1	42.7 \pm 1.4	21.9 \pm 1.7
117.0	10	21.1 \pm 0.6	32.4 \pm 2.7	43.7 \pm 1.9	22.6 \pm 1.7
234.0	10	21.0 \pm 1.2	32.9 \pm 3.0	43.0 \pm 2.0	22.1 \pm 1.8

Tab1-3 the BW & BW increase of mice in hemolysis void spot test. (mean \pm SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	21.3 \pm 0.6	32.8 \pm 3.0	42.2 \pm 1.9	20.9 \pm 2.3
58.5	10	20.9 \pm 1.1	32.7 \pm 2.7	42.8 \pm 2.3	21.9 \pm 2.6
117.0	10	21.2 \pm 0.8	33.4 \pm 2.7	42.2 \pm 2.6	21.0 \pm 2.1
234.0	10	21.3 \pm 0.9	33.2 \pm 2.1	43.8 \pm 3.1	22.6 \pm 3.0

Tab1-4 the BW & BW increase of mice in serum hemolysin test. (mean \pm SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
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Normal control	10	20.9±0.8	32.3±2.7	41.8±2.5	20.9±2.4
58.5	10	21.0±0.8	32.4±1.6	43.0±2.5	22.0±2.7
117.0	10	21.2±0.9	32.3±2.0	43.1±2.0	22.0±2.6
234.0	10	20.4±1.0	32.6±2.0	43.4±2.8	23.0±3.0

Tab1-5 the BW & BW increase of mice in carbon clearance test. (mean±SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	20.2±1.0	32.0±3.5	42.6±2.9	22.4±2.7
58.5	10	20.6±1.1	32.3±2.8	43.1±2.4	22.5±2.3
117.0	10	19.9±1.4	32.2±2.7	43.6±2.1	23.7±2.5
234.0	10	20.1±0.8	32.7±2.7	44.0±2.2	23.9±2.4

Tab1-6 the BW & BW increase of mice in abdominal cavity macrophage phagocytize test. (mean±SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	20.0±1.1	32.4±3.3	41.7±4.0	21.7±3.9
58.5	10	20.4±1.0	32.9±2.6	43.6±2.5	23.2±2.8
117.0	10	20.4±0.7	32.4±3.0	43.7±2.4	23.3±2.6
234.0	10	20.3±1.1	33.1±2.1	43.8±2.4	23.5±2.1

Tab1-7 the BW & BW increase of mice in NK cytoactive test. (mean±SD)

Dose group (mg/kg)	Animal number	Initial BW (g)	BW on 14d (g)	BW on 28d (g)	BW increase (g)
Normal control	10	20.1±0.5	24.6±1.3	27.5±1.7	7.4±2.0
58.5	10	20.1±1.0	24.3±1.1	27.4±1.6	7.3±1.8
117.0	10	20.2±1.0	25.1±1.3	28.1±2.1	7.9±2.3
234.0	10	19.9±1.2	25.2±1.0	28.2±1.0	8.4±1.6

Tab2. the effect of the JianShenBao Capsule of FeiLing Brand contents on the body weight, the ratio of spleen weight / body weight (SW/BW), the ratio of thymus weight / body weight (TW/BW) of mice (mean±SD)

Dose group (mg/kg)	Animal number	SW/BW (mg/g)	TW/BW (mg/g)
Normal control	10	2.68±0.24	1.57±0.23
58.5	10	2.65±0.31	1.70±0.24
117.0	10	2.60±0.33	1.85±0.24
234.0	10	2.62±0.15	1.64±0.32

2.2 The test of ConA induced spleen lymphocyte conversion in mice (MTT method)

The difference vale result of the ConA Hole Optical Density—No ConA Hole Optical Density of each group in the test of ConA induced spleen lymphocyte conversion in mice (MTT method) is given in Tab3.

Tab3. the effect of the JianShenBao Capsule of FeiLing Brand contents on the mice lymphocyte multiplication capacity (mean \pm SD)

Dose group (mg/kg)	Animal number	ConA Hole Optical Density—No ConA Hole Optical Density
Normal control	10	0.010 \pm 0.007
58.5	10	0.012 \pm 0.006
117.0	10	0.017 \pm 0.012
234.0	10	0.022 \pm 0.013*

* Dunnett's test, compared with the normal control group, shows significant difference, $P < 0.05$.

Treat the difference of ConA Hole Optical Density—No ConA Hole Optical Density in Tab3 with variance analysis, and then find out significant difference ($F=3.05$, $P < 0.05$) among groups. After the Dunnett's test, compared with the normal control group, the difference of ConA Hole Optical Density—No ConA Hole Optical Density of the JianShenBao Capsule of FeiLing Brand contents group of 234mg/kg dosage shows significant difference ($P < 0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice lymphocyte multiplication capacity at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).

2.3 Dinitrofluorobenzene (DNFB) induced mice lagged allergy test (ear tumefaction method)

The weight difference vale result of the L-Ear-Piece—R-Ear-Piece of each group in the DNFB induced mice lagged allergy test is given in Tab4.

Tab4. the effect of the JianShenBao Capsule of FeiLing Brand contents on the mice lagged allergy (mean \pm SD)

Dose group (mg/kg)	Animal number	difference of the ear-piece-weight (mg)
Normal control	10	30.9 \pm 4.1
58.5	10	27.3 \pm 9.5
117.0	10	37.2 \pm 2.7
234.0	10	43.4 \pm 5.7*

* Dunnett's test, compared with the normal control group, shows significant difference, $P < 0.05$.

Treat the difference of the ear-piece-weight in Tab4 with variance analysis, and then find out very significant difference ($F=13.61$, $P < 0.01$) among groups. After the Dunnett's test, compared with the normal control group, the weight difference of L-Ear-Piece—R-Ear-Piece of the JianShenBao Capsule of FeiLing Brand contents group of 234mg/kg dosage shows significant difference ($P < 0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can reinforce the action of the mice T-lymphocyte multiplying to sensitized lymphocyte at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).

2.4 Antibody genesis cell detection test (Jerne improved glassslide method)

The result of hemolysis void spot number of each group in the antibody genesis cell detection test is given in Tab5.

Tab5. the effect of the JianShenBao Capsule of FeiLing Brand contents on the number of the mice antibody genesis cell (mean \pm SD)

Dose group (mg/kg)	Animal number	hemolysis void spot number ($1/10^6$ spleen cells)
Normal control	10	252 \pm 28.2
58.5	10	268 \pm 27.8
117.0	10	296 \pm 51.0

234.0

10

382 ± 58.1*

* Dunnett's test, compared with the normal control group, shows significant difference, $P < 0.05$.

Treat the difference of the hemolysis void spot number in Tab5 with variance analysis, and then find out very significant difference ($F=17.79$, $P < 0.01$) among groups. After the Dunnett's test, compared with the normal control group, the difference of the hemolysis void spot number of the JianShenBao Capsule of FeiLing Brand contents group of 234mg/kg dosage shows significant difference ($P < 0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can increase the number of the mice antibody genesis cell at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).

2.5 Serum hemolysin determination (blood coagulation method)

The result of mice serum hemolysin antibody accumulation index of each group in the serum hemolysin determination test is given in Tab6.

Tab6. The effect of the JianShenBao Capsule of FeiLing Brand contents on mice serum hemolysin antibody accumulation index (mean ± SD)

Dose group (mg/kg)	Animal number	Antibody accumulation index #
Normal control	10	1.685 ± 0.240 (48.4 ± 1.7)
58.5	10	2.139 ± 0.148* (137.7 ± 1.4)
117.0	10	2.203 ± 0.132* (159.6 ± 1.4)
234.0	10	2.300 ± 0.123* (199.5 ± 1.3)

The antibody accumulation index is a mean of LOG, which in the () is a geometrical mean.

* Dunnett's test, compared with the normal control group, shows significant difference, $P < 0.05$.

Treat the antibody accumulation index in Tab6 with variance analysis, and then find out very significant difference ($F=26.58$, $P < 0.01$) among groups. After the Dunnett's test, compared with the normal control group, the difference of the antibody accumulation index of each of three JianShenBao Capsule of FeiLing Brand contents groups of shows significant difference ($P < 0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can promote the generation of mice antibody at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).

2.6 Carbon clearance test in mice

The result of the mice carbon clearance ability of each group in the mice carbon clearance test is given in Tab7.

Tab7. The effect of the JianShenBao Capsule of FeiLing Brand contents on mice carbon clearance ability (mean ± SD)

Dose group (mg/kg)	Animal number	Phagocytic index
Normal control	10	7.53 ± 1.18
58.5	10	7.77 ± 0.92
117.0	10	8.20 ± 1.13
234.0	10	8.82 ± 1.08*

* Dunnett's test, compared with the normal control group, shows significant difference, $P < 0.05$.

Treat the phagocytic index in Tab7 with variance analysis, and then find out significant difference ($F=2.93$, $P < 0.05$) among groups. After the Dunnett's test, compared with the normal control group, the phagocytic index of the JianShenBao Capsule of FeiLing Brand contents group of 234mg/kg dosage shows significant difference ($P < 0.05$), which indicate that the contents of the JianShenBao

Capsule of FeiLing Brand can reinforce the carbon clearance ability of mice at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).

2.7 The mice abdominal cavity macrophage phagocytizing cock red blood cell test. (half in vivo method)

The result of the phagocytic rate & phagocytic index of the mice abdominal cavity macrophage phagocytizing cock red blood cell of each group in the mice abdominal cavity macrophage phagocytizing cock red blood cell test is given in Tab8.

Tab8. the effect of the JianShenBao Capsule of FeiLing Brand contents on the phagocytosis ability of mice abdominal cavity macrophage (mean \pm SD)

Dose group (mg/kg)	Animal number	Phagocytic rate (%)	Phagocytic index
Normal control	10	18.6 \pm 4.1	0.226 \pm 0.052
58.5	10	21.4 \pm 4.1	0.284 \pm 0.055
117.0	10	27.6 \pm 6.0*	0.334 \pm 0.081*
234.0	10	33.6 \pm 7.3*	0.416 \pm 0.101*

* Dunnett's test, compared with the normal control group, shows significant difference, $P<0.05$.

Treat the phagocytic rate & phagocytic index in Tab8 with variance analysis, and then find out very significant difference ($F=14.45$, $P<0.01$; $F=11.64$, $P<0.01$) among groups. After the Dunnett's test, compared with the normal control group, the phagocytic rate & phagocytic index of each of the JianShenBao Capsule of FeiLing Brand contents groups of 117mg/kg and 234mg/kg dosage shows significant difference ($P<0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can reinforce the phagocytosis ability of mice abdominal cavity macrophage at the dosages of 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 10, 20 times of the commended dosage used for human body).

2.8 NK cytoactive determination test [lactate dehydrogenase (LDH) determination method]

The result of the mice NK cytoactive of each group in the NK cytoactive determination test is given in Tab9.

Tab9. the effect of the JianShenBao Capsule of FeiLing Brand contents on the mice NK cytoactive (mean \pm SD)

Dose group (mg/kg)	Animal number	NK cytoactive (%)
Normal control	10	9.1 \pm 5.3
58.5	10	34.3 \pm 16.0*
117.0	10	40.1 \pm 15.5*
234.0	10	47.9 \pm 15.0*

* Dunnett's test, compared with the normal control group, shows significant difference, $P<0.05$.

Treat the NK cytoactive in Tab9 with variance analysis, and then find out very significant difference ($F=15.00$, $P<0.01$) among groups. After the Dunnett's test, compared with the normal control group, the NK cytoactive of each of three JianShenBao Capsule of FeiLing Brand contents groups shows significant difference ($P<0.05$), which indicate that the contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice NK cytoactive at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).

3. CONCLUSION

- 3.1 After the oral injection in the mice with the contents of the JianShenBao Capsule of FeiLing Brand, at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body), no adverse effect on body weight, the ratio of spleen weight / body weight, the ratio of thymus weight / body weight of mice has been found out.
- 3.2 The test of ConA induced spleen lymphocyte conversion in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice lymphocyte multiplication capacity at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
- 3.3 The result of the dinitrofluorobenzene (DNFB) induced lagged allergy test in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the action of the mice T-lymphocyte multiplying to sensitized lymphocyte at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
- 3.4 The test result of the antibody genesis cell detection has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can increase the quantity of the mice antibody genesis cell at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
- 3.5 The test result of the serum hemolysin determination has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can promote the generation of mice antibody at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).
- 3.6 The result of the carbon clearance test in mice has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the carbon clearance ability of mice at the dosage of 234mg/kg, bw/d (corresponding to 20 times of the commended dosage used for human body).
- 3.7 The result of the mice abdominal cavity macrophage phagocytizing cock red blood cell test has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the phagocytosis ability of mice abdominal cavity macrophage at the dosages of 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 10, 20 times of the commended dosage used for human body).
- 3.8 The test result of the NK cytoactive determination has indicated, that the tested contents of the JianShenBao Capsule of FeiLing Brand can reinforce the mice NK cytoactive at the dosages of 58.5mg/kg, bw/d, 117mg/kg, bw/d, 234mg/kg, bw/d (corresponding to 5, 10, 20 times of the commended dosage used for human body).

According to the result judgement principle of the Immunoregulation Function Test Method in the < Health Food Function Evaluation Program & Test Method >, the JianShenBao Capsule of FeiLing Brand manufactured by Wuxi Feiling Biological SciTech Co. Ltd. has the immunoregulation function.

NO CONTENT WITHIN THIS PAGE

Test operator_____

Reviser_____

Chief of the Lab_____

Seal of the test institution

Auditor_____

Signed & Issued by_____

August 31, 2002

Report on Test & Identification

(2002) No. weijianzi 132

P1 of 1 pages

Sample submitter: Wuxi Feiling Biological SciTech Co. Ltd.

Sample name: Fruiting Body and Fungus Species inoculated on inclined-plane-culture-medium

Sample quantity: 1

Submission date: July, 2002

Chief of the Test & Identification (signature):

Operator of the Test & Identification

(signature):

Zhou Xin Guang

He Wen Hua

The content and result of the Test & Identification: (This Identification Result is only valid to the submitted sample. The name of the identification side shouldn't be used for business promotion without owner's permission.)

Character of the Fruiting Body:

The fruiting body is of middle size, with a stalk, and has the texture as cork. The pileus has the shape of kidney or round, which is 13cm wide and 1.5cm thick. At the initial stage, the pileus is brilliant saffron yellow and its edge is white, and then it will change into rufous and have the paint-like brightness after aged. There are circulated ribs and radialized wrinkles on its upper surface, and its edge is thin and entad curled. The flesh is white to light brown, 1.5cm thick. The pore-surface is white initially, and then will change into light brown, and then brown. The pores are brown, 1cm long. The pore-orifices are round, and there are 3 to 5 orifices per millimeter. The stalk is cylindrical, pleurogenous, 6.5-10.5cm long, 2cm wide, violet brown with brightness.

The hypha system is a trisomy. The procreation hypha is transparent, with thin wall, ramose, and the diameter is 3.5 μ m. The framework hypha is light yellow, with thick wall (and the hypha is almost solid), with tree-like branches, the diameter of trunk is 3 μ m. The flagelliform colorless swirly hypha is formed at the end of the branch, with thick wall, many curls, branches, and the diameter is 2.0 μ m, most form into bovista type swirly hypha.

Character of the spore:

The spore is brown, egg type, 9.6 \times 4.8-7.2 μ m. After top truncation, the doublelayer wall is shown. The outer wall is smooth and the inter wall is spinulate.

Denomination: *Ganoderma lucidum*

Remarks: The submitted sample for identification has been preserved in the Fungus Species Preservation Room of the Institute of Microbiology, Chinese Academy of Sciences (IMCAS). The serial number: 2002-8. The preservation period is two years.

The School of Public Health, Southeast University
Nanjing Public Health Prevention Medical Institute

REPORT ON QUALITY TEST

(Food) Test No. 20020021-3

Sample Name Refined Flavor and Full Flavor of ganoderma
lucidum of Feiling Brand

Sample Submitter Wuxi Feiling Biological SciTech Co., LTD.

Sample Producer Wuxi Feiling Biological SciTech Co., LTD.

August 31, 2002

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Test Report of Nanjing Research Institute of Public Preventive Medicine

Sample Name Refined Flavor and Full Flavor of ganoderma lucidum of Feiling Brand
Test Classification on commission
Sample Submitter Wuxi Feiling Biological SciTech Co., LTD.
Telephone +86(510)2709489
Address of the Submitter 118 Jiankang Road, Wuxi, Jiangsu, P.R. of China
Zip Code 214028
Sampling Spot 118 Jiankang Road, Wuxi, Jiangsu, P.R. of China
Date of Sample Received July 1st, 2002
Batch No. or Date of production 20010717 (refined flavor), 20020327 (full flavor)
Physical Property of the Sample both brown powder
Submitting Personnel Mao Jian
Amount of the Sample 50 grams each

On commission of Wuxi Feiling Biological SciTech Co., Ltd., our institute performed the Sarcoma S-180 Suppression Test of the product samples of the Refined Flavor and Full Flavor of ganoderma lucidum of Feiling Brand produced by the Wuxi Feiling Biological SciTech Co., LTD., in July and August of 2002. The results are listed as below:

1. Materials and methods

1.1 Samples The Feiling Brand Refined Flavor and Full Flavor of ganoderma lucidum produced by Wuxi Feiling Biological SciTech Co., Ltd. Both samples were brown powder. The recommended uptake dosage for human is 11.7 mg/kg·bw/d (0.7~1.4g/d, 0.7g/d counted; the body weight is estimated 60kg.).

1.2 Mice with tumor loaded Provided by Shanghai Institute of Materia Medica.

1.3 Experimental Mice Seventy male ICR mice, body weights ranged from 18 to 22 grams, were provided by the Experimental Animal Center of Nanjing Medical University, License: SCXK (Jiangsu) 2002-0015. License for animal usage: SYXK (Jiangsu) 2002-0057. The mice were divided into one blank control group (accept 0.5% amylum solution), 3 testing groups of Feiling Brand refined flavor at dosages of 58.5mg/kg.bw/d, 117.0 mg/kg.bw/d and 234 mg/kg.bw/d, 3 testing groups of Feiling Brand full flavor at dosages of 58.5mg/kg.bw/d, 117.0 mg/kg.bw/d and 234 mg/kg.bw/d. The dosages were equivalent to 5 folds, 10 folds and 20 folds of the recommended human uptake dosage of 11.7 mg/kg.bw/d, respectively. The samples of refined and full flavor of the ganoderma lucidum were all prepared to be the suspension with 0.5% amylum solution (cooled after being boiled). All the experimental mice were fed with full rate granular feeding stuff, free to ingestion of food and water.

1.4 Methods The mice were poured the suspension into their stomachs at the volume dosage of 0.4ml/20g·bw, once per day. On the 20th day, the mice were aseptically inoculated 0.2ml of the S-180 sarcoma cell suspension [25%(V/V), diluted with normal saline], on the right front leg subcutaneously. Stop the daily stomach pouring 10 days after the inoculation,

another 3 days after which, kill the mice with the method of neck vertebra disjoints. Perform the decollement of the tumor. Weigh the tumors after drying the surface with filter paper.

1.5 Data Statistics Manage the data in each group with the method of the single factor analysis of variance, using the statistical software of SAS 6.12.

2. Results

2.1 Body Weights

The data in Table 1-1 and Table 1-2 show the body weights varying situation of the mice. The results suggest that no significant difference ($p>0.05$) between the body weights of the mice in each testing-dosage group of Refined Flavor and Full Flavor of ganoderma lucidum of Feiling Brand and that of the mice in the blank control group.

Table 1-1 The body weights of the mice in the sarcoma suppression test with the Refined Flavor

Dosage (mg/kg)	Number of Mice	Initial Body Weight (g)	Body Weight on Day 20 (g)	Body Weight on Day 33* (g)
Blank Control	10	20.1 ± 0.9	33.2 ± 2.8	29.6 ± 2.1
58.5	10	20.0 ± 1.1	32.2 ± 2.1	31.0 ± 2.1
117.0	10	20.1 ± 1.0	31.9 ± 3.2	29.8 ± 2.5
234.0	10	20.3 ± 0.9	32.9 ± 3.2	30.3 ± 2.3

*the weight of the tumor body excluded

Table 1-2 The body weights of the mice in the sarcoma suppression test with the Full Flavor

Dosage (mg/kg)	Number of Mice	Initial Body Weight (g)	Body Weight on Day 20 (g)	Body Weight on Day 33* (g)
Blank Control	10	20.1 ± 0.9	33.2 ± 2.8	29.6 ± 2.1
58.5	10	20.8 ± 1.0	32.0 ± 3.0	29.9 ± 4.4
117.0	10	20.0 ± 1.0	33.4 ± 2.7	29.8 ± 2.9
234.0	10	19.8 ± 0.9	32.2 ± 2.7	30.4 ± 2.3

*the weight of the tumor body excluded

2.2 The Sarcoma S-180 Suppression Test

The data of tumor weights of mice in each group of the sarcoma S-180 suppression test are listed in Table 2-1 and Table 2-2.

Table 2-1 The weights of the sarcoma S-180 in the sarcoma suppression test with the Refined Flavor
(average value \pm standard deviation)

Dosage (mg/kg)	Number of Mice	Tumor weight (g)
Blank Control	10	1.68 ± 0.26
58.5	10	$1.29 \pm 0.29^*$
117.0	10	$1.19 \pm 0.31^*$
234.0	10	$0.97 \pm 0.39^*$

*Dunnett's T Test, significantly different from that of the blank control group

The results of the variance test on the data in Table 2-1 indicated the significant differences between every two groups ($F=8.63$, $p<0.001$). The results of the Dunnett's Test indicated the significant differences ($p<0.05$) between the tumor weights of mice in the blank control group and that of the mice in the refined flavor-sample groups with dosages of 58.5mg/kg, 117mg/kg and 234mg/kg, which suggested that the refined flavor of ganoderma lucidum of Feiling Brand was effective on suppression of mice Sarcoma S-180 at the dosages of 58.5mg/kg, 117mg/kg and 234mg/kg (equivalent to the 5, 10 and 20 folds of the recommended human uptake dosage).

Table 2-2 The weights of the sarcoma S-180 in the sarcoma suppression test with the Full Flavor
(average value \pm standard deviation)

Dosage (mg/kg)	Number of Mice	Tumor weight (g)
Blank Control	10	1.68 ± 0.26
58.5	10	$1.11 \pm 0.34^*$
117.0	10	$1.02 \pm 0.25^*$
234.0	10	$0.78 \pm 0.28^*$

*Dunnett's T Test, significantly different from that of the blank control group

The results of the variance test on the data in Table 2-1 indicated the significant differences between every two groups ($F=17.61$, $p<0.001$). The results of the Dunnett's Test indicated the significant differences ($p<0.05$) between the tumor weights of mice in the blank control group and that of the mice in the full flavor-sample groups with dosages of 58.5mg/kg, 117mg/kg and 234mg/kg, which suggested that the full flavor of ganoderma lucidum of Feiling Brand was effective on suppression of mice Sarcoma S-180 at the dosages of 58.5mg/kg, 117mg/kg and 234mg/kg (equivalent to the 5, 10 and 20 folds of the recommended human uptake dosage).

3. Conclusion

3.1 No adverse effect on the mice body weight was observed after the stomach-pouring of the refined or full flavor of the ganoderma lucidum of Feiling Brand at the dosages of 58.5mg/kg, 117mg/kg and 234mg/kg (equivalent to the 5, 10 and 20 folds of the recommended human uptake dosage).

3.2 The results of Sarcoma S-180 Suppression Test indicated that the samples of the refined or full flavor of the ganoderma lucidum of Feiling Brand were effective on the suppression of the mice sarcoma S-180, at the dosages of 58.5mg/kg, 117mg/kg and 234mg/kg (equivalent to the 5, 10 and 20 folds of the recommended human uptake dosage).